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Propriétés physico-chimiques et impact environnemental de liquides ioniques

Physico-chemical properties and environmental impact of ionic liquids

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Abstract

Ionic liquids are the salts composed only by bulky, unsymmetrical and flexible organic cations and inorganic or organic anions. Their melting points are particularly low, usually below 100 ℃. The ionic liquids present several interesting properties: high thermal stability, low vapor pressure, non-flammability and tunable properties by changing the anion or cation. They are considered as promising high performance fluids with low environmental impact that can be applied in the fields of chemistry, chemical engineering or materials science both in processes (separation, catalysis) or as devices (optical components or lubricants).

The application of ionic liquids at an industrial scale is still limited and fundamental information and reliable data on their properties, environmental fate and impact are rare. In this context and for the development of applications in wide scale, the research on ionic liquids with an expected low environmental impact (less toxic, readily biodegradable) is essential. The introduction of oxygenated functional groups – ester, ether or hydroxyl – in the alkyl side chains of imidazolium and pyridinium-based ionic liquids is expected to greatly improve their biodegradability. The effect of the functionalization on the physico-chemical properties of the ionic liquids is important in order to propose efficient, low environmental impact, ionic liquids for different applications and chemical processes.

In this work, we have selected different ionic liquids based on the imidazolium, pyridinium, pyrrolidinium and ammonium cations, with or without functional groups (hydroxyl/ester/ether), and with three types of anions. Our objective was to examine if the modification of chemical structures of the ions effectively have lower environmental impact, and if they their interesting properties are remained.

We have studied, for all the ionic liquids, several physico-chemical properties considered important to quantify the environmental impact of chemicals: the density, the viscosity, the gas solubility, the aqueous solubility, the octanol-water partition coefficient and the aqueous diffusivity. We have also tested their toxicity towards four different microorganisms and their biodegradation in presence of pure strain of bacteria. Finally, we have tried to develop some empirical and semi-empirical models based on molecular

structure information for predicting some of these properties.

The introduction of oxygenated groups in the alkyl chains on cations does not change significantly the volumetric properties of ionic liquids, or their diffusion coefficient in water, but increases the viscosity of the pure salts up to one order of magnitude. Carbon dioxide solubilities in ionic liquids are not significantly influenced by the introduction of oxygen functional groups in the cations of the ionic liquids except in the case of the pyridinium based ionic liquids for which the carbon dioxide solubility decreases significantly due to a defavourable entropic contribution to the Gibbs energy of solvation.

The modification of the ionic liquids by introducing oxygenated chemical functions makes them more biodegradable. In the case of imidazolium-based ionic liquids, the presence of the ester group makes the cation more susceptible to hydrolysis, the imidazolium ring being still resistant to the degradation. The functionalization of the cation also increases the solubility in water of the resulting ionic liquids and reduces the octanol-water partition coefficient and their toxicity, leading us to conclude that they are more environmental friendly than the non-functionalized ionic liquids.

Keywords: ionic liquids, oxygenated groups, physico-chemical properties, environmental impact

Résumé

Les liquides ioniques sont des composés uniquement constitués d'ions souvent volumineux, asymétriques et flexibles. Ils possèdent des températures de fusion basses, typiquement au-dessous de 100 °C. Ils sont considérés comme des solvants prometteurs pour une chimie durable du fait, notamment, de leur tension de vapeur négligeable. Ils présentent d'autres propriétés intéressantes: grande stabilité thermique, pression de vapeur quasiment nulle, non-inflammabilité, propriétés modulables par changement de l'anion ou du cation, *etc*. Ils sont classiquement organisés par familles, en fonction de la structure chimique de leur cation: imidazolium, pyridinium, pyrrolidinium, *etc*. Leurs domaines d'application sont très variés: synthèse, catalyse, séparation et extraction.

Cependant, l'utilisation des liquides ioniques à l'échelle industrielle est encore limitée par manque de connaissances fondamentales et par l'absence de données fiables sur leurs propriétés, leur devenir et leur impact environnementaux. La toxicité des liquides ioniques a récemment été démontrée ainsi qu'une résistance à la biodégradation. Dans ce contexte et en vue du développement à grande échelle de l'utilisation de ces composés, la recherche de liquides ioniques à faible impact environnemental (moins toxiques, facilement biodégradables) devient essentielle.

L'introduction dans les chaînes latérales alkyles des imidazolium et pyridinium de groupements fonctionnels oxygenés, qui peuvent être reconnus par des enzymes (*ex*. esters, éthers, alcools terminaux), a grandement amélioré leur biodégradabilité. En même temps, il est important de garantir que la présence de ces groupements n'affecte pas les propriétés physico-chimiques des liquides ioniques requises pour un usage spécifique dans des procédés chimiques.

Dans ce projet de doctorat, nous avons sélectionné divers liquides ioniques basés sur les cations imidazolium, pyridinium, pyrrolidinium et ammonium, avec ou sans groupements fonctionnels (alcool/ester/éther) et avec trois types d'anions, comme cibles d'étude. L'objectif était d'examiner si les modifications de structures peuvent effectivement baisser leur impact environnmental tout en gardant leurs propriétés

intéressantes pour des applications industrielles.

Dans un premier temps, nous avons étudié plusieurs propriétés pysico-chimiques importantes pour des applications ultérieures et/ou indicatrices de leur impact environnemental : les propriétés volumiques, la viscosité, la solubilité de gaz, la solubilité dans l'eau, le coefficient de partage octanol-eau et le coefficient de diffusion dans l'eau. Dans un deuxième temps, nous avons étudié leur impact environnemental par la mesure de la toxicité sur quatre microorganismes différents et l'étude de leur biodégradation en présence de souches pures de bactéries. En complément, nous avons essayé de trouver des modèles basés sur les informations structurales pour estimer certaines propriétés.

L'insertion de groupements oxygénés sur la chaîne alkyle des cations ne change pas significativement les propriétés volumiques; ni le coefficient de diffusion dans l'eau. Les propriétés de solvatation des liquides ioniques basés sur des cations imidazolium et ammonium ne sont pas modifiées significativement, mais celles des pyridinium sont réduites, à cause d'une contribution entropique défavorable à l'énergie de Gibbs de solvatation. La présence de groupements oxygénés dans la chaîne alkyle du cation augmente la viscosité d'un ordre de grandeur comparativement aux liquides ioniques sans groupements oxygénés. Dans le cas de l'anion octylsulfate la viscosité augmente de deux ordres de grandeur. La présence de groupements oxygénés augmente la biodégradabilité des liquides ioniques. La presence de groupements esters rend les liquides ioniques plus sensibles à l' hydrolyse dans les conditions abiotiques et biotiques, toutefois le noyau imidazolium n'en devient pas biodégradable pour autant. L'introduction de groupements oxygénés augmente la solubilité dans l'eau, diminue la valeur du coefficient de partage octanol-eau et entraine une baisse de la toxicité ce qui signifie que ces liquides ioniques présentent un plus faible impact environnemental.

Mots-clés: ioniques liquides, groupements oxygénés, propriétés pysico-chimiques, impact environnmental

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1. INTRODUCTION

1.1 Introduction in English

An ionic liquid is a salt composed only by ions and whose melting point is below some arbitrary temperature, usually 100 ℃. The ionic liquids contain bulky, unsymmetrical and flexible organic cations and inorganic or organic anions.[1,2] The large ions make the electrostatic interactions weaker. The asymmetry of the structure lowers the lattice energy, and hence their melting points are particularly low.[3,4]

Different ionic liquids can be formed by appropriately combining cations and anions or by chemically modifying either the cation or the anion. At least 10^6 ionic liquids can be synthesized,[2,5,6] while there are only hundreds of traditional organic solvents. Due to the modular nature of ionic liquid, their properties can readily be tuned for many specific applications in different important chemical processes, reported for example in the areas of catalysis, biocatalysis, synthetic chemistry and electrochemistry.[2] They are often called "designer solvents".[6]

Some of the most commonly employed cations and anions in ionic liquids formation are shown in Figure 1.1.[2,5]

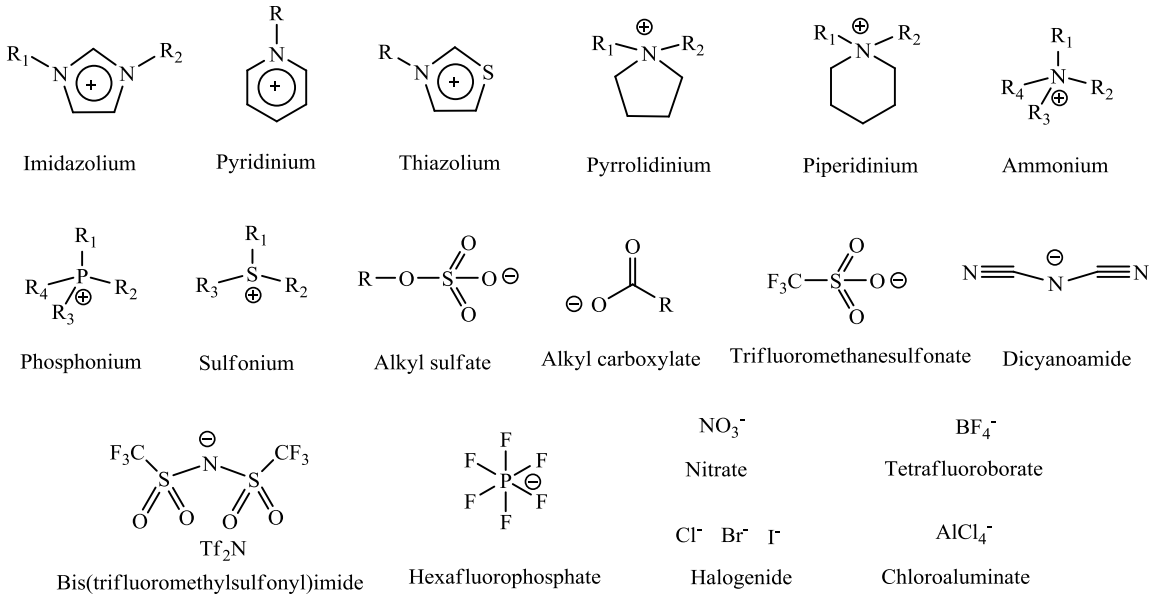


Figure 1.1. Cations and anions commonly employed in ionic liquids formation.

Although the electrostatic bonds of ionic liquids are generally weaker than those of the

normal salts, the interactions between ions are still strong enough to cause very low vapour pressures under ambient conditions. Experimental studies and molecular simulations have shown nanostructuration of ionic liquids into polar and non-polar domains, which influences the way how other molecules and ions are solvated in these media.[7]

The negligible volatility means no potential release of vapour in the environment and no inhalation risks to the manipulators, so the ionic liquids are often considered as potential "green" solvents to replace traditional volatile organic solvents.[6] Because of their remarkable properties, ionic liquids have captured a quickly rising interest and simulated the imagination of chemists, engineers, often bridging academic and industrial communities in the last decades.[8,9]

The negligible volatility alone cannot make ionic liquids green solvents. The low toxicity, high biodegradability, low bioaccumulation, and low environment impact are important for the use of ionic liquids in different applications.

While lipases play an important role in the metabolism of fatty acids, non-specific esterases protect cells by hydrolysing any ester linkages encountered in foreign molecules.[10] Correspondingly, Gathergood and Scammells[11,12] demonstrated that the introduction of esters into the alkyl side chain of N-methylimidazolium ionic liquids significantly improves their biodegradability. Harjani *et al.*[13] have proved that the introduction of ester in the alkyl side chains of pyridinium-based ionic liquids also can greatly improve their biodegradability. The pyridinium[14] and ammonium[15] based ionic liquids have also shown a better biodegradability than the non-functionalized imidazolium-based ionic liquids.

1.1.1 Objectives

The first objective of this thesis is to examine if the oxygenated functionalized ionic liquids really show a lower environmental impact than the non-functionalized ones. We aim to provide information on the effects of different structures as well as some functional groups in various ionic liquids on their toxicity, biodegradation, bioaccumulation, and environmental impact.

So far, the information on the physico-chemical properties of the modified ionic

liquids is rather limited. Therefore, the second objective of this thesis is to study how the modification(s) of the molecular structure affects their physico-chemical properties. As the measurement of all the properties of the enormous amount of possible ionic liquids would be quite fastidious, we try to establish relationships between molecular structures and certain pertinent physico-chemical properties of different ionic liquids families.

This study is expected to contribute to the development of really "green solvents" with interesting properties for their applications.

1.1.2 Strategy

Ionic liquids of various families, functionalized by oxygenated groups such as ester, ether or hydroxyl groups, and non-functionalized ones were studied for comparison. This work mainly focuses on different types of cations: two most used cations- imidazolium and pyridinium, which have aromatic rings, along with pyrrolidinium that has a non-aromatic ring, and ammonium that has no ring. The effect of different anions has been also studied: bis(trifluoromethylsulfonyl)imide $[Tf_2N]^-$, octylsulfate $[C_8SO_4]^-$ and acetate $[OAc]^-$. $[Tf_2N]^-$ is favorable for reducing the viscosity[16,17] and increases the solubility of CO_2.[18] Some ionic liquids with $[C_8SO_4]^-$ showed comparably higher biodegradability.[10,19] $[OAc]^-$ is very promising because it is non-toxic and environmental benign. Some imidazolium-based ionic liquids containing $[OAc]^-$ have been proved to allow a high CO_2 solubility.[20] The ionic liquids studied are constituted of the combination of the cations and anions presented in Figure 1.2.

In the following sections, for the convenience of writing and reading, acronyms are used for the ionic liquids cations presented in this thesis. The cation head groups were abbreviated as: ''Im'' for imidazolium, ''Py'' for pyridinium, ''Pyr'' for pyrrolidinium, "Pip" for piperidinium, "Gu" for guanidinium, "Qu" for quinolinium, "Mor" for morpholinium, "P" for phosphonium and ''N'' for ammonium. The acronym of one side chains attached to the head group adopted the modified "Simplified Molecular Input Line Entry System" (SMILES)[21]: from the first atom attached to the nitrogen, atoms are represented by their atomic symbols and the hydrogen attached to the carbon and nitrogen

are elided. The lower case numbers indicate the number of successive identical atoms. Ester group is COO whereas hydroxyl group is OH. The other alkyl chains were abbreviated by the first letter of the alkyl name. For example, the 1-butyl-3-methylimidazolium moiety was denoted as $[C_4mIm]^+$. For ammonium and phosphonium-based ionic liquids, all the alkyl chains attached to nitrogen or phosphor atom as are indicated by the numbers of carbon atoms in the chains in lower case form.

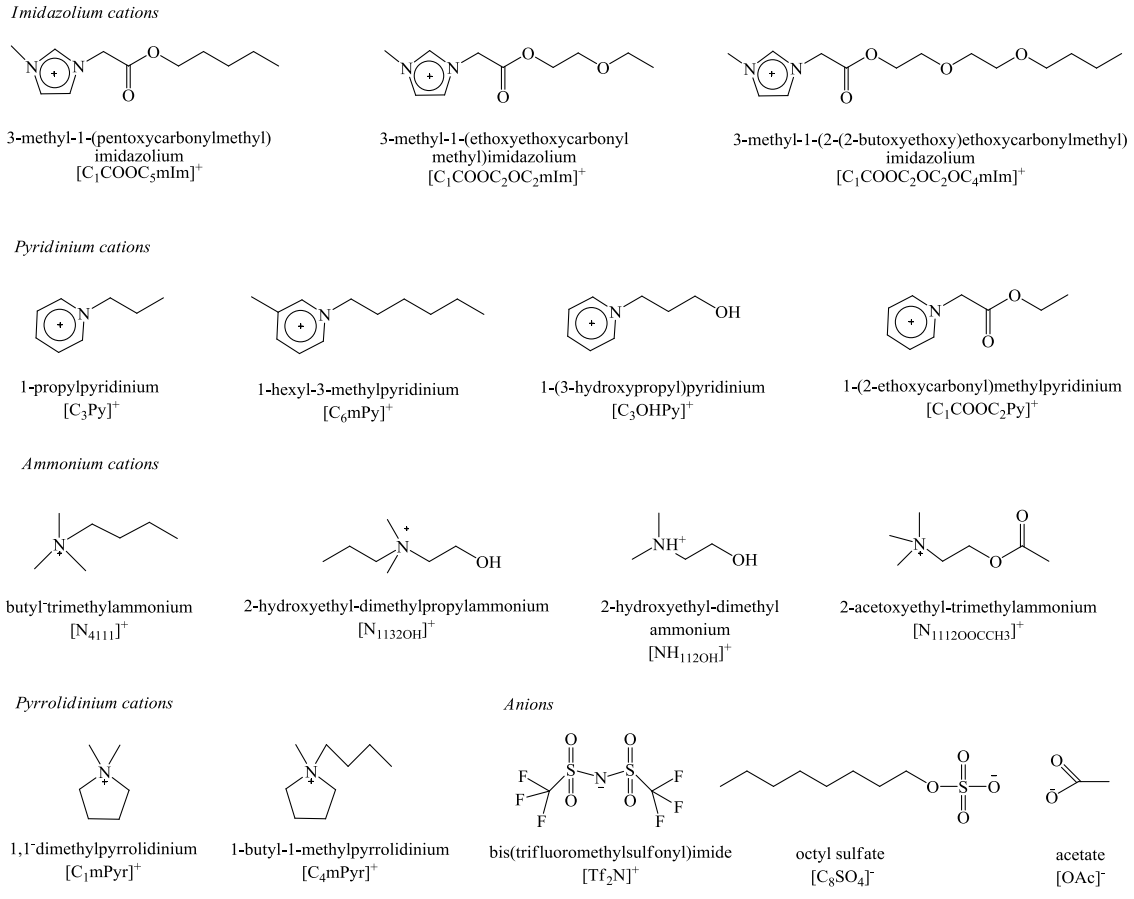


Figure 1.2. Abbreviations, structures and names of ions forming the studied ionic liquids.

We have measured several parameters either significant for the potential applications or for the evaluation of environmental impact, or important for both. For applications, density (ρ), viscosity (η) and gas solubility (CO_2, x_{CO2}, C_2H_6, x_{C2H6}, CH_4, x_{CH4}) were selected. For environmental impact, both physico-chemical properties - aqueous solubility (x_{IL}), octanol-water partition coefficient (K_{OW}), diffusion coefficient in water (D_{AB}), and biological properties - toxicity, biodegradability are studied. The physico-chemical

properties experimentally measured for each ionic liquid are not always the same and they are listed in Table 1.1.

Table 1.1. Experimentally measured physico-chemical properties of the studied ionic liquids.

Ionic liquids	physico-chemical properties							
	ρ	η	x_{CO2}	x_{C2H6}	x_{CH4}	D_{AB}	x_{IL}	K_{ow}
Imidazolium-based ionic liquids								
$[C_1COOC_5mIm][C_8SO_4]$	√	*l*[a]	√	√	√	√	√	√
$[C_1COOC_5mIm][Tf_2N]$	√	*l*	√	√	√	√	√	√
$[C_1COOC_2OC_2mIm][Tf_2N]$	√	*l*	√	√		√	√	√
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	√	*l*	√	√		√	√	√
Pyridinium-based ionic liquids								
$[C_3Py][Tf_2N]$[b]						√	√	√
$[C_6mPy][Tf_2N]$	√	√	*l*			√	√	√
$[C_3OHPy][Tf_2N]$	√	√	√			√	√	√
$[C_1COOC_2Py][Tf_2N]$	√	√	√			√	√	√
Ammonium-based ionic liquids								
$[N_{4111}][Tf_2N]$	√	√	√			√	√	*n*[c]
$[N_{1132OH}][Tf_2N]$	√	√	*l*			√	√	*n*
$[N_{1112OOCCH3}][Tf_2N]$	√	√	√			√	√	*n*
$[NH_{112OH}][Ac]$	√	√	*l*			√	√	*n*
Pyrrolidinium-based ionic liquids								
$[C_1mPyr][Tf_2N]$[b]						√	√	*n*
$[C_4mPyr][Tf_2N]$	√	√	*l*			√	√	*n*

[a] *l* = reported in literature; [b] solid at t < 60 ℃; [c] *n* = unable to be detected by our available equipment

Based on the experimental results, we have tried to build some empirical structure-property models which can predict the property of ionic liquids. Three simple models to respectively estimate density, octanol-water partition coefficient and diffusion coefficient in water are proposed.

1.1.3 Organization of the manuscript

This manuscript is constituted by a section describing the scientific context of this work referring to the applications and some physico-chemical properties of ionic liquids; review on the published studies about toxicity, biodegradability assays and biodegradation of ionic liquids; a collection of five articles, published or submitted; a section including some unpublished results and conclusion.

In the introduction, ionic liquids are presented and the objectives and strategy followed in this thesis are discussed. In the section presenting the scientific context, some properties of ionic liquids as the density, the solvation properties, and the octanol-water partition coefficient are explained. Transport properties are also pertinent to define the environmental impact of ionic liquids and we present an overview of the viscosity and molecular diffusion in water of different ionic liquids. Octanol-water partition coefficient is more detailed than other properties because it is relatively complex but less commonsensible in spite of its importance, and the techniques of measuring other properties in our laboratory are already fairly mutual but the measurement of octanol-water partition coefficient was set out for the first time. The bibliographic part overviews the recent research results on the toxicology and biodegradation of ionic liquids.

Five articles are produced from my thesis work: (1) on the physico-chemical properties of imidazolium-based ionic liquids, published in *ChemSusChem*, 2010, 3, 377 – 385; (2) on the octanol-water partition coefficient of imidazolium-based ionic liquids, published in proceeding EQIFASE 2009; (3) on the toxicity, biodegradability and environmental impact of imidazolium-based ionic liquids, submitted in *J. Hazard. Mater*.; (4) on the physico-chemical properties of pyridinium, ammonium and pyrrolidinium-based ionic liquids, submitted in *J. Chem. Eng. Data*; (5) on the environmental impact of pyridinium, ammonium and pyrrolidinium-based ionic liquids, submitted in *Chemosphere*.

1.2 Introduction en Français

Les liquides ioniques sont des sels constitués uniquement d'ions, dont le point de fusion est inférieur à une température arbitraire, souvent 100 °C. Ils sont constitués de cations organiques volumineux et d'anions organiques ou inorganiques, ce qui leur confère une asymétrie de forme et de charge ainsi qu'une grande flexibilité.[1,2] La taille importante de ces ions rend les interactions électrostatiques faibles et leur cohésion est abaissée par l'asymétrie de la structure, ce qui entraine des points de fusion particulièrement faibles.[3,4]

Différents liquides ioniques peuvent être formés soit par la combinaison appropriée de cations et d'anions soit par la modification chimique du cation ou de l'anion. Ceci offre un choix de combinaisons considérables, la littérature rapporte aujourd'hui la synthèse possible d'au moins 10^6 liquides ioniques différents,[2,5,6] par opposition avec les quelques centaines de solvants organiques traditionnels existants. Le fait de pouvoir choisir tel ou tel ion pour former un liquide ionique permet de moduler leurs propriétés. Ils peuvent ainsi être utilisés pour de nombreuses applications spécifiques dans d'importants processus chimiques se rapportant aux domaines de la catalyse, de la biocatalyse, de la chimie synthétique et électrochimie,[2] et sont souvent désignés par le terme de «designer solvents».[2,6] Les cations et les anions les plus couramment utilisés dans la formation des liquides ioniques sont présentés dans la Figure 1.3.[2,5]

Bien que les liaisons électrostatiques des liquides ioniques soient généralement plus faibles que celles des sels normaux, les interactions entre les ions restent suffisamment fortes pour générer des pressions de vapeur très faibles dans les conditions ambiantes. Les études expérimentales et les simulations moléculaires ont montré que la nanostructuration des liquides ioniques dans les domaines polaires et non-polaires influence la manière dont les autres molécules et les ions sont solvatés dans ces milieux.[7]

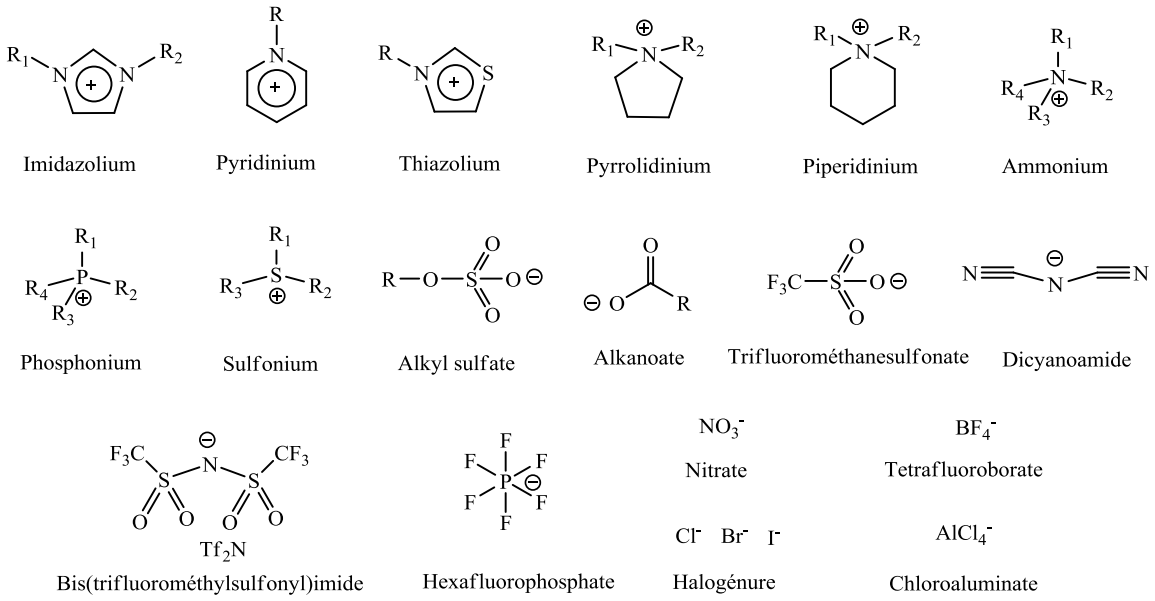


Figure 1.3. Cations et anions communément utilisés dans la formation des liquides ioniques.

En raison de la volatilité négligeable des liquides ioniques, il n'y a pas de rejet de vapeur dans l'environnement et pas de risque d'inhalation par les utilisateurs. Par conséquent, les liquides ioniques sont souvent considérés comme des solvants potentiellement «verts» par opposition aux solvants organiques traditionnels volatils.[6] Du fait de leurs propriétés remarquables, l'intérêt des liquides ioniques a rapidement augmenté et a stimulé l'imagination des chimistes et des ingénieurs, renforçant le lien entre les communautés académiques et industrielles dans les dernières décennies.[8,9]

La volatilité négligeable des liquides ioniques n'en fait pas à elle seule des solvants verts. La faible toxicité, la haute biodégradabilité, la faible bioaccumulation, et le faible impact environnemental sont autant d'arguments pour l'utilisation des liquides ioniques dans différents domaines d'application.

Les travaux de Gathergood et Scammells[10,11,12] ont montré que l'introduction d'un groupement ester sensible à l'hydrolyse enzymatique dans la chaîne d'alkyle du cation méthylimidazolium améliore grandement la biodégradabilité de ces composés. Le changement de la nature de l'anion a aussi son importance, l'utilisation d'anions alkylsulfates par exemple, contribue également à réduire l'impact environnemental. Harjani *et al.*[13] ont prouvé que l'introduction d'une fonction ester dans la chaîne alkyle de liquides

ioniques basés sur un cation pyridinium peut également améliorer considérablement leur biodégradabilité. Les liquides ioniques basés sur des cations pyridinium[14] ou ammonium[15] ont montré une meilleure biodégradabilité que les liquides ioniques basés sur des cations imidazolium non fonctionnalisés.

1.2.1 Objectifs

Le premier objectif de cette thèse est de déterminer si les liquides ioniques fonctionnalisés avec des groupements oxygénés ont réellement un impact environnemental moindre que celui des liquides ioniques non fonctionnalisés. Nous avons donc étudié les effets du changement de structure du cation, et de l'anion, et/ou de l'ajout de certains groupes fonctionnels sur la toxicité, la biodégradation, la bioaccumulation et l'impact environnemental des liquides ioniques.

Jusqu'à présent, les informations existant sur les propriétés physico-chimiques des liquides ioniques fonctionnalisés sont plutôt limitées. Par conséquent, le deuxième objectif de cette thèse est de vérifier si la modification de la structure moléculaire entraine un changement significatif de ces propriétés physico-chimiques. Du fait de l'impossibilité de mesurer toutes les propriétés de tous les liquides ioniques existants, nous avons établi des relations entre les structures moléculaires et les propriétés physico-chimiques les plus pertinentes de différentes familles de liquides ioniques.

Cette étude doit contribuer dans une certaine mesure à accélérer le développement de vrais solvants verts présentant des propriétés intéressantes.

1.2.2 Stratégie

Des liquides ioniques de différentes familles, non fonctionnalisés ou fonctionnalisés avec des groupements esters, éthers ou hydroxyles ont été étudiés comparativement. Le présent travail se concentre plus particulièrement sur l'influence du type de cation. Sont étudiés les cations possédant des cycles aromatiques comme les cations imidazolium et pyridinium, les cations avec un cycle non aromatique comme le pyrrolidinium ou encore des cations sans cycle comme l'ammonium. L'influence de la nature de l'anion a également

été prise en compte. Pour cela, trois anions différents ont été étudiés: le bis (trifluorométhylsulfonyl)imide $[Tf_2N]^-$, l'octylsulfate $[C_8SO_4]^-$ et l'acétate $[OAc]^-$. La présence de l'anion $[Tf_2N]^-$ entraine une réduction de la viscosité[16,17] et une augmentation de la solubilité du CO_2.[18] Certains liquides ioniques avec l'anion $[C_8SO_4]^-$ ont montré une biodégradabilité élevée.[12,19] L'anion $[OAc]^-$ est très prometteur car il est non-toxique pour l'environnement. Certains liquides ioniques ayant pour anion l'acétate et basés sur un cation de type imidazolium permettent une grande solubilité du CO_2.[20] L'ensemble des cations et des anions constituant les liquides ioniques étudiés sont représentés dans la Figure 1.4.

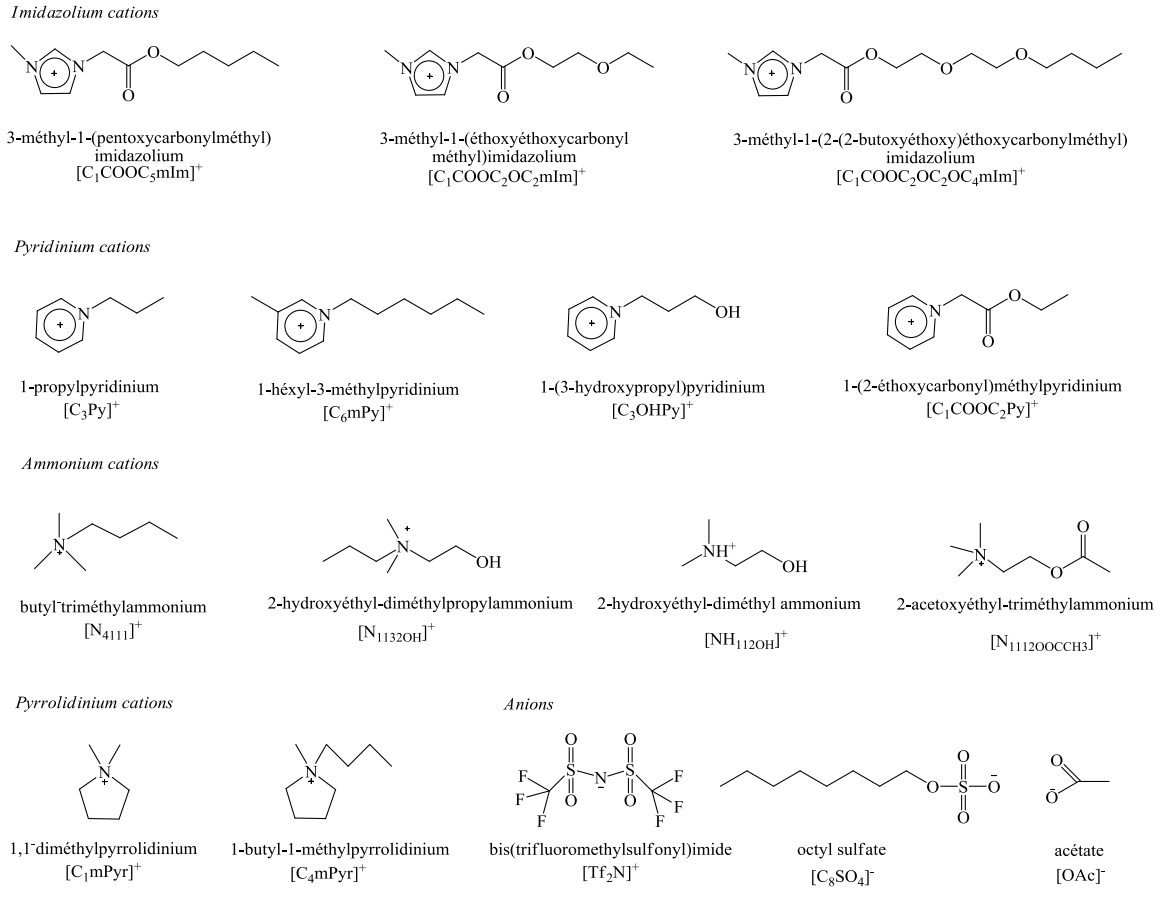


Figure 1.4. Abréviations, structures et noms des ions constituant les liquides ioniques étudiés.

Dans les sections suivantes, par commodité d'écriture et de lecture, les acronymes

suivants vont être utilisés pour designer les différents cations présentés dans cette thèse. Les groupes de tête des cations sont abrégés de la manière suivante : "Im" pour imidazolium," Py" pour pyridinium, "Pyr" pour pyrrolidinium, "Pip" pour pipéridinium, "Gu" pour guanidinium, "Qu" pour quinolinium, "Mor" pour morpholinium, "P" pour phosphonium et "N" pour ammonium. De la même manière, les chaînes latérales fixées au groupement principal adoptent le "Simplified Molecular Input Line Entry System" (SMILES)[21]: en prenant comme point de départ le premier atome fixé à l'azote, les atomes sont représentés par leur symbole atomique mais sans prendre en compte l'hydrogène fixé sur le carbone ou l'azote. Le nombre figurant à côté de chaque symbole indique le nombre de mêmes atomes successifs. Un groupement ester s'écrit COO tandis qu'un groupement hydroxyle s'écrit OH. Les autres chaînes alkyles sont abrégées par la première lettre du nom du groupement alkyle. Par exemple, la cation 1-butyl-3-méthylimidazolium est noté $[C_4mIm]^+$. Pour les liquides ioniques basés sur des cations ammonium et phosphonium, les chaînes alkyles fixées à l'azote ou au phosphore sont désignées par le nombre d'atomes de carbones en indice.

Plusieurs paramètres importants d'un point de vue application, évaluation de l'impact environnemental, ou les deux à la fois ont été mesurés. Dans le domaine des applications, les propriétés telles que la masse volumique (ρ), la viscosité (η) et la solubilité des gaz (CO_2, x_{CO2}, C_2H_6, x_{C2H6}, CH_4, x_{CH4}) ont été choisies. Au niveau de l'impact environnemental, des propriétés physico-chimiques comme la solubilité dans l'eau (x_{IL}), le coefficient de partage octanol-eau (K_{OW}), le coefficient de diffusion dans l'eau (D_{AB}) mais aussi des propriétés biologiques comme la toxicité et la biodégradabilité ont été étudiés. Les propriétés physico-chimiques mesurées expérimentalement ne sont pas les mêmes pour tous les liquides ioniques étudiés et sont listées dans le tableau 1.2.

En se basant sur les résultats expérimentaux, des modèles empiriques structure-propriété ont été établis afin de prédire les propriétés des liquides ioniques. Trois modèles pour estimer respectivement la masse volumique, le coefficient de partage octanol-eau et le coefficient de diffusion dans l'eau sont proposés.

Tableau 1.2. Propriétés physico-chimiques mesurées expérimentalement pour les différents liquides ioniques étudiés.

Liquides Ioniques	Propriétés physico-chimiques							
	ρ	η	x_{CO2}	x_{C2H6}	x_{CH4}	D_{AB}	x_{IL}	K_{ow}
Liquides ioniques de la famille imidazolium								
$[C_1COOC_5mIm][C_8SO_4]$	√	*l*[a]	√	√	√	√	√	√
$[C_1COOC_5mIm][Tf_2N]$	√	*l*	√	√	√	√	√	√
$[C_1COOC_2OC_2mIm][Tf_2N]$	√	*l*	√	√		√	√	√
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	√	*l*	√	√		√	√	√
Liquides ioniques de la famille pyridinium								
$[C_3Py][Tf_2N]$[b]						√	√	√
$[C_6mPy][Tf_2N]$	√	√	*l*			√	√	√
$[C_3OHPy][Tf_2N]$	√	√	√			√	√	√
$[C_1COOC_2Py][Tf_2N]$	√	√	√			√	√	√
Liquides ioniques de la famille ammonium								
$[N_{4111}][Tf_2N]$	√	√	√			√	√	*n*
$[N_{1132OH}][Tf_2N]$	√	√	*l*			√	√	*n*
$[N_{1112OOCCH3}][Tf_2N]$	√	√	√			√	√	*n*
$[NH_{112OH}][Ac]$	√	√				√	√	*n*
Liquides ioniques de la famille pyrrolidinium								
$[C_1mPyr][Tf_2N]$[b]						√	√	*n*
$[C_4mPyr][Tf_2N]$	√	√	*l*			√	√	*n*

[a] *l* = rapporté dans littérature; [b] solide à t < 60 °C; [c] *n* = ne peut être détectée par nos équipements disponibles

1.2.3 Organisation du manuscrit

Ce manuscrit est constitué de la manière suivante : une introduction, un chapitre décrivant le contexte scientifique de ce travail et comprenant les applications possibles et certaines propriétés physico-chimiques des liquides ioniques, une troisième partie faisant l'état des lieux de la bibliographie existant sur la toxicité et la biodégradation des liquides ioniques, puis une partie regroupant une collection de cinq articles, publiés ou soumis avant de finir par un chapitre regroupant certains résultats non publiés ainsi que la

conclusion.

L'introduction présente les liquides ioniques, les objectifs et la stratégie suivie dans cette thèse.

La seconde partie porte sur le contexte scientifique et décrit certaines propriétés des liquides ioniques telles que la densité, les propriétés de solvatation, le coefficient de partage octanol-eau, les propriétés de transport (afin de mettre en évidence leur impact environnemental), la viscosité et la diffusion moléculaire dans l'eau. Le coefficient de partage octanol-eau est plus détaillé que les autres propriétés car il est relativement complexe et moins courant en dépit de son importance, et les techniques de mesure des autres propriétés de notre laboratoire sont mieux connues alors que le coefficient de partage octanol-eau est mesuré pour la première fois au LTIM. Une étude bibliographique regroupant les résultats des recherches récentes sur la toxicologie et la biodégradation de liquides ioniques a été réalisée. Cinq articles ont été écrits à partir des résultats obtenus durant la thèse: (1) un premier sur les propriétés physico-chimiques des liquides ioniques basés sur un cation de type imidazolium, celui-ci a été publié dans *ChemSusChem*, 2010, 3, 377 – 385; (2) un second sur le coefficient de partage octanol-eau des liquides ioniques basés sur un cation de type imidazolium; (3) un troisième sur la toxicité, la biodégradabilité et l'impact environnemental des liquides ioniques fonctionnalisés ou non-fonctionnalisés basés sur un cation de type imidazolium, soumis dans *J. Hazard. Mater*.; (4) un quatrième sur les propriétés physico-chimiques des liquides ioniques basés sur des cations de type pyridinium, ammonium et pyrrolidinium, soumis dans *J. Chem. Eng. Data*; (5) et un dernier sur l'impact environnemental des liquides ioniques basés sur des cations de type pyridinium, ammonium et pyrrolidinium, soumis dans *Chemosphere*.

2. Scientific Context

2.1 Applications of ionic liquids

Since Paul Walden first reported the synthesis of a low melting-point salt (ethylammonium nitrate) in 1914,[22] ionic liquids have not been the object of active researches for several decades. However, the field of ionic liquids is growing at a surprising rate in the recent ten years.

At present, ionic liquids are finding more and more applications in the domain of catalysis, biocatalysis, synthetic chemistry, electrochemistry, and a variety of analytical processes, such as, separation, extraction, electroanalysis, sensing, and spectrometry. A number of detailed reviews have introduced the applications as well as the advantages of using ionic liquids as novel solvents.[6,23,24,25,26]

Plechkova and Seddon[6] have predicted that the ionic liquids will be used commercially in all the fields illustrated in Figure 2.1, because of their tunable properties through their endless diversity. At the present time, only about 300 ionic liquids are commercially available, which means there is a large space for development.

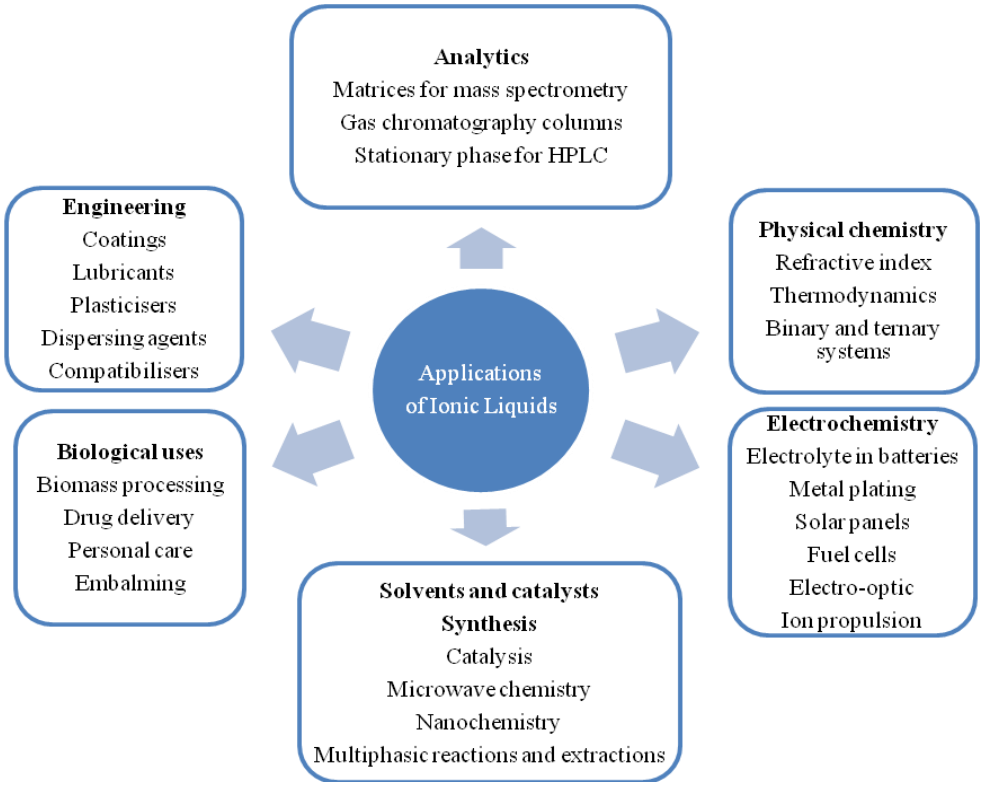


Figure 2.1. Causeway to the future applications of ionic liquids (according to Plechkova and Seddon[6]).

2.2 Fate of ionic liquids in the environment

It is important to study the environmental impact of ionic liquids before using them at a large industrial scale.

For convenience, the environment can be divided into distinct yet interconnected compartments or phases, lithosphere (soil or land surface), hydrosphere (water), atmosphere (air), and biosphere (biota).[27, 28] According to their physico-chemical properties, chemicals can be transported within and transferred between the different compartments (Figure 2.2).

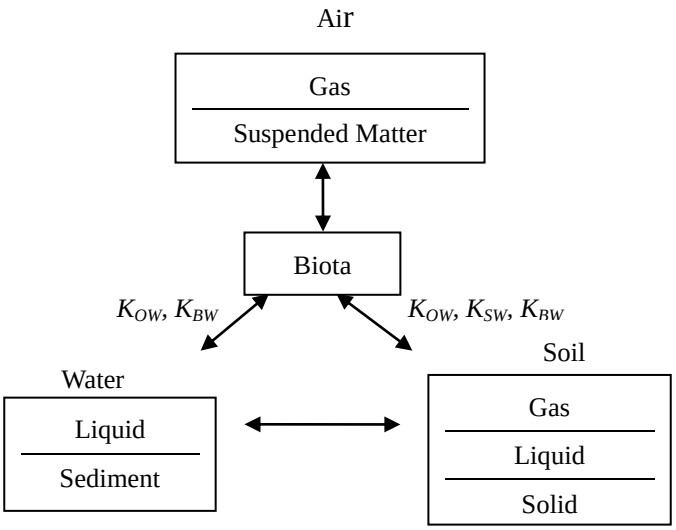


Figure 2.2. Partitioning and transport of chemicals in environmental compartments. K_{OW} = octanol-water partition coefficient, K_{SW} = soil-water partition coefficient, K_{BW} = biota-water partition coefficient (according to Sangster[28]).

The physico-chemical properties important to determine the environment partitioning of chemicals are: boiling point, melting point, gas and liquid densities, surface tension, vapour pressure, aqueous solubility, air-water partition coefficient, sorption coefficient for soil and sediment, bioconcentration factor. Besides, diffusivity in air and water, phase transfer coefficient for air-soil and phase transfer coefficient for air-water are key parameters to determine their environmental transport and fate.[28]

Because of their negligible vapour pressure, ionic liquids generally will not be present in the atmosphere, which minimizes contamination and manipulators' health risk. Nevertheless, most of them have significant solubility in water and can enter into the environment via this medium, either by release or by accidents. They will be then transported and transferred into the different environment compartments depending on

their physico-chemical properties. They can also undergo chemical and/or biological transformations leading to the formation of degradation products or to their mineralization (complete degradation into CO_2, H_2O, *etc*.).

In the lithosphere. In a general way, the organic compounds can be present in soil air, soil water and soil solids (Figure 2.3). The ionic liquids do not distribute in soil air because they are not volatile.

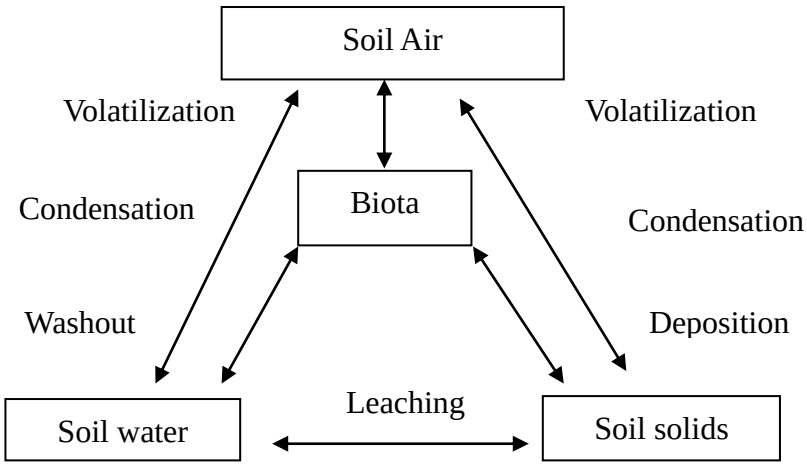


Figure 2.3. The fate of chemicals in the soil column (according to Sangster[28]).

Their retention and mobility in soils are strongly influenced by their tendency to be sorbed onto the various components of the soil matrix (in particular clay minerals and/or organic matter) or to dissolve in soil water. Several mechanisms can be involved in the sorption process depending on the soil characteristics and on the ionic liquid physico-chemical properties. Stepnowski [29] first evidenced that electrostatic and hydrophobic interactions were the major contributors to the sorption of 1-alkyl-3-methyl imidazolium cations on soil and sediments. A more detailed study[30] with selected natural soils containing different organic content, cation exchange capacity and particle size distribution showed the influence of the ionic liquid concentration: at low concentration (0-1 mmol.L^{-1}), the strength of the sorption was positively correlated with the length of the alkyl chain regardless on soil type. The same conclusion was described by Matzke *et al.*[31]; at higher concentration (> 1 mmol.L^{-1}), multilayered sorption was observed with the formation of admicelles, leading to a sorption capacity above the theoretical sorption threshold.

The main contributor in soils for ionic liquid sorption remains controversial: a primarily correlation of the sorption strength with the exchange cation capacity and therefore with the clay particle fraction was found by Stepnowski[30] whereas other authors[32,33] showed increased sorption capacity with higher soil or sediment organic matter content. In fact, both types of soil components increase the sorption (and decrease desorption) of the ionic liquids as shown by the addition of clay and organic matter in the work of Matzke *et al*.[31] According to the surface properties of the solid matrix, electrostatic interactions of the imidazolium cations with the negatively charged sites of clay minerals[31] or organic matter,[32,33] hydrophobic interactions[28,29] or even hydrogen bondings[32] are supposed to be the major mechanism of ionic liquid sorption process. The anion can also play a crucial role in the ionic liquid sorption: Ionic liquid with $[BF_4]^-$ as anion showed higher sorption capacity compared to the same one with $[Tf_2N]^-$, indicating the strong ionic pair in the soil matrix[31].

In conclusion, hydrophilic ionic liquids tend to dissolve in water but they may also sorb onto soils. Lipophilic ionic liquids tend to be strongly sorbed to the soil solids, restricting their movement and their availability to soil organisms. As a consequence, they show little tendency to be leached down through the soil profile by water.

In the hydrosphere. Hydrophilic ionic liquids will dissolve in and distribute throughout surface water. The dissolved chemicals spread out in water, due to velocity shear within the rivers or advection associated with water currents within the lakes, as well as due to diffusion, caused by random motion of molecules. The dense ionic liquids may settle themselves whereas light ionic liquids can be found in the surface of water, *e.g.*, dissolved in surface oil film. They can also sorb onto dissolved or suspended particles, in particular organic matter by different mechanisms: ion exchange, hydrogen bondings, hydrophobic interactions[33]. For example, 1-butyl- and 1-octyl-3-methylimidazolium chloride associated strongly with dissolved organic matter reducing the bioavailability of these ionic liquids and therefore their toxicity[34]. These ionic liquids are easily sorbed onto suspended particles, which ultimately settle to the bottom sediment.

In the biosphere. All living organisms are involved, at different levels, in the recycling of organic and inorganic matter in the environment. Nevertheless, due to their capacities to colonize all the biotopes and their metabolic adaptabilities, microorganisms play a key role

in the degradation of xenobiotic compounds. The first criterion that has to be taken into account is the bioavailability of the compound. As shown previously, lipophilic ionic liquids tend to be strongly sorbed to the soil solids, restricting their availability to soil microorganisms. Hydrophilic ionic liquids tend to dissolve in water but they may also sorb onto suspended particles reducing their bioavailabilty. In some cases microorganisms can develop strategies to overcome the poor availability, such as biofilm construction and biosurfactant production. After uptake in microorganism, xenobiotics enter metabolism pathways that modify the chemical structure of the molecule; these reactions often act to detoxify poisonous compounds.

The fate of the uptake compounds can be as follows:

- no degradation but bioaccumulation of the parent molecule. For example, accumulation of $[P_{666(14)}][Tf_2N]$ has been shown in the membrane of *Escherichia coli* cells.[35]

- partial degradation into metabolites which accumulate in the environment and can be more toxic or more persistent than the parent molecule. For example, imidazolium ring is resistant to biodegradation.[12]

- complete mineralization. For example, some of the choline-based ionic liquids are easily biodegraded.[2]

Terrestrial plants can remediate, treat, stabilise or control contaminated substrates by physiological processes and are thereby used to decontaminate soils. These physiological processes include plants and associated microorganism metabolisms. Toxicities of different ionic liquids towards plants have been described in monocotyledonous and dicotyledonous plants but few data is available concerning phytoremediation of soils contaminated by ionic liquids.

Likewise, a number of publications highlight the toxic effects of ionic liquids to aquatic biological ecosystems but the metabolic pathways involving in their potential transformation have been rarely investigated, and studies concerning bioaccumulation among the food chain are also lacking.

The ionic liquids cannot be uptaken by inhalation, so ingestion and passive diffusion are the main sources of uptake. Passive diffusion is the spontaneous diffusion of substances

across a membrane. A balance between the solubility of ionic liquids in lipids and in water, usually indicated by the octanol-water partition coefficient, is crucial for predicting the passive diffusion.

After uptake, lipophilic ionic liquids tend to be associated with lipoproteins and membranes of blood cells. They can be stored in lipophilic (hydrophobic) environments, especially fat depots. Especially dangerous, lipophilic ionic liquids can cross the blood-brain barrier membrane and enter the brain. Some highly lipophilic ionic liquids can be excreted to a limited extent, like for other pollutants, in milk by mammals or in eggs by birds, reptiles, and invertebrates (Figure 2.4).

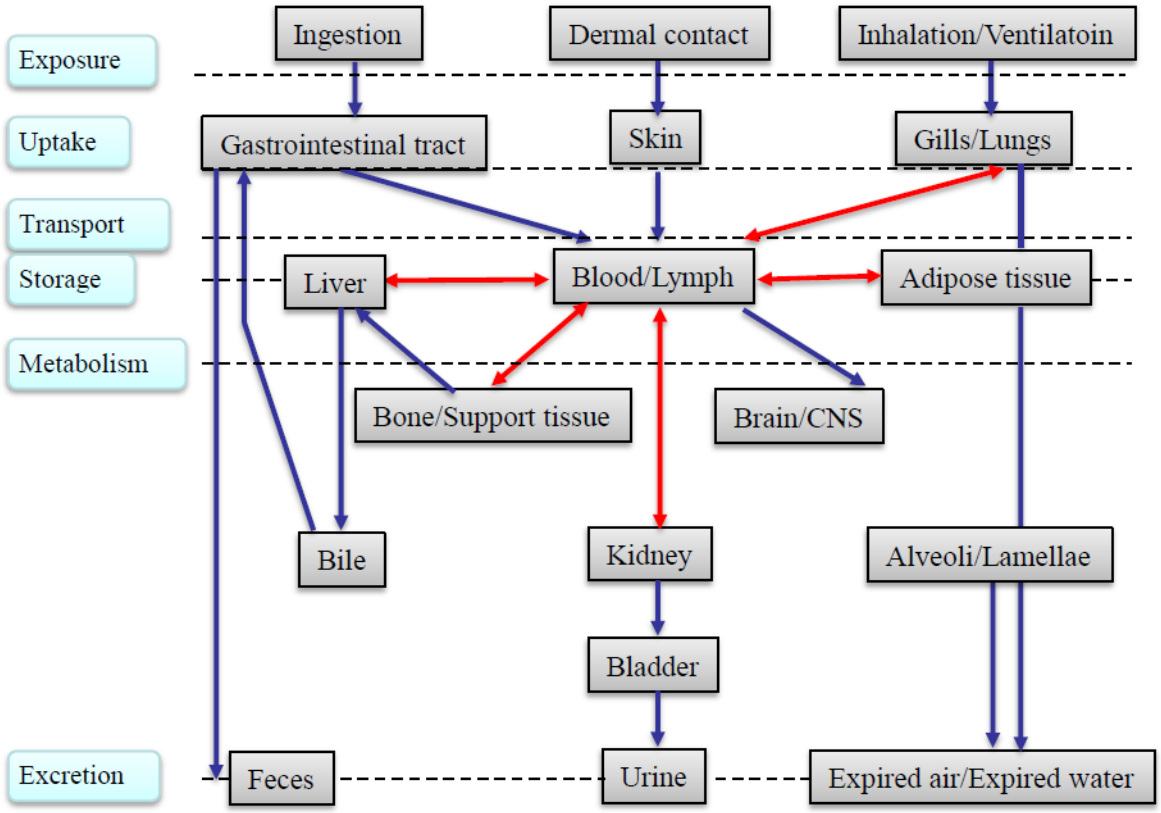


Figure 2.4. Xenobiotic's fate in vertebrate (according to Yuan[36]).

Conversely, hydrophilic ionic liquids have more tendencies to dissolve in blood water than to associate with lipoproteins and membranes. The water-soluble ionic liquids are readily excreted in feces or urine.

Toxicity and biodegradation of ionic liquids will be overviewed in the Section 3.

2.3 Physico-chemical properties of ionic liquids

Physical chemistry is an underlying aspect providing the insights into the molecular factors that determine the properties of ionic liquids.[37]

The physico-chemical properties of ionic liquids can be tailored to the requirements of a chemical or technological process.[2] Normally, many ionic liquids are designed to have low combustibility, non-flammability, excellent thermal stability, favorable solvation properties for many organic and inorganic materials, high ionic conductivity, low volatility, wide temperature range as a liquid, and a wide electrochemical window.[2]

When searching the best ionic liquids for a particular purpose, physico-chemical data that are essential for engineering processes have to be accessed.[37,38] The knowledge of the properties of ionic liquids is still limited despite of their more and more frequent use.[39] It is important to identify trends and to relate the physico-chemical properties to the molecular structures of the cations and anions as the enormous variety of possible ionic liquids pertains the mere screening of their properties.

The density and the transport properties are important to develop processes involving ionic liquids.[40] The solvation properties of ionic liquids are key information directly useful for different applications in different domains ranging from catalysis to separations. Gas solubility data can also serve to access the structure of the liquid and to identify particular solvation sites. Gas solutes have often a simple and well known molecular structure and can serve as probes to map the structure of ionic liquid solvents.[18,41]

Density, transport and solvation properties of ionic liquids have been chosen as peculiar and important properties and will be briefly presented in this chapter.

2.3.1 Density

Density is an important property for process engineering and equipment design for the application of ionic liquids. Additionally, density is easy to determine with a good reproducibility, and can be significantly affected by impurities,[42] so it is usable to determine the purity of ionic liquids. Densities of ionic liquids are often measured using a U-shape vibrating-tube densimeter operating in a static mode and the density is related to

the square of measured period of vibration.[43]

A considerable amount of experimental data is available on the density as a function of the temperature and pressure for a range of imidazolium, pyridinium, ammonium, phosphonium, and pyrrolidinium-based ionic liquids. The densities of ionic liquids at ambient conditions generally range from 0.8 to 3.3 $g.cm^{-3}$, while for classic solvents it varies from 0.6 to 1.7 $g.cm^{-3}$.[6]

For a given anion, ionic liquid densities decrease with increasing the length of the alkyl side chains on the imidazolium ring.[39,44] The cations containing ether or hydroxyl groups form ionic liquids with higher densities.[45] Anions of higher molar masses generally lead to higher density ionic liquids with the same imidazolium cation, except in the case of anions with long alkyl chains.[44,45]

On the basis of the published data, some research groups have concluded that the density as a function of the temperature and pressure of a range of ionic liquids can be quantitatively estimated using a group contribution scheme – the molar volumes of different ionic liquids being calculated from the sum of the effective molar volumes of the constituent groups.[46] Based on this conception, Jacquemin *et al.*[39] proposed a group contribution model for the calculation of ionic liquid molar volumes and densities with an estimated uncertainty lower than 0.4%.

The knowledge on the density as a function of temperature and of pressure allows the calculation of the mechanical coefficients[39] - volume expansivities and isothermal compressibilities - that can provide information on fluid structure and molecular interactions.[47] The isothermal compressibility of ionic liquids is comparable with that of water and lower than that of organic solvents.[43] The model of Jacquemin *et al.* has been extended to temperatures up to 423K and pressures to 207MPa[48] and allows the prediction of the density of several families of ionic liquids with uncertainties lower than 0.5%. The model also allows the calculation of the mechanical coefficients to within $\pm 20\%$.

2.3.2 Solvation properties

A. Gas solubility

Ionic liquids attract increasing interest because they are regarded as probable

replacement of traditional organic solvents. For the moment, an important part of the ionic liquids applications involves the reaction of permanent or condensable gases such as hydrogenations, hydroformylations, and oxidations.[18,49] Furthermore, gas storage, gas separation, and the use of gases to separate solutes from ionic liquid solutions show a great potential.[49]

To explore the possibilities of further uses of ionic liquids, it is important to understand the underlying solvent behavior at the molecular level. Canongia *et al.*[7,37] have shown that it exists a microphase segregation exists between polar and nonpolar domains in the ionic liquids that determines most of their properties and in particular the way in which solvation can be understood in these media.

Gas solubility as a function of temperature provides important information about the solute-solvent interactions, and about the structure of the solutions, through the assessment of the thermodynamic properties of solution. Furthermore the link between the macroscopic properties and the microscopic interactions can then be established, and the molecular mechanisms of solvation can be easily investigated by molecular simulation.[50,51]

Different solutes - nonpolar, polar, and associating - will be solvated in distinct local environments and will interact preferably with certain parts of the individual ions.[37] Carbon dioxide interacts preferentially with the polar domains of the ionic liquids in region of its quadrupole moment.[51]

In our laboratory, the solubility of gases in ionic liquids has been studied experimentally at low-pressures using an isochoric saturation technique.[51,52] In this technique, a known amount of gaseous solute is put in contact with a precisely determined quantity of degassed solvent at a constant temperature inside an accurately known volume. When thermodynamic equilibrium is attained at a given temperature, the pressure above the liquid solution is constant and is directly related to the solubility of the gas in the liquid.

Carbon dioxide is the most studied gaseous solute. The values in the literature for the Henry's law constants are all in accord within approximately ± 10%,[52] while there are several methods to measure gas solubility: volumetric, gravimetric and spectroscopic methods.[5]

The molecular interactions between ionic liquids and different types of chemicals can be relatively easier understood from the studies of gas solvation, which is useful to predict the interactions of ionic liquids with various substances in environment.

B. Solubilities in water

The solubility of ionic liquids in water (water solubility or aqueous solubility) is an important parameter that determines the chemical's environmental impact and fate. The distribution of chemicals in different environmental components, the toxicity,[53] the bioavailability, and the bioaccumulation is directly related to the hydrophobicity or lipophilicity of the compound.[54] Furthermore, aqueous solubility is also essential for designing and developing ionic liquids for liquid-water extraction processes, which ionic liquids with low aqueous solubility are required.[55,56,57]

The aqueous solubilities of ionic liquids can be measured by different experimental methods, *eg*. UV-spectroscopy[58], thermogravimetry[58], Karl-Fisher titration[59], electrospray ionization mass spectrometry[60] and potentiometry with ion-selective electrodes[61].

The aqueous solubilities are characterized by a highly pronounced anion effect. Some anions render the ionic liquids fully miscible with water at ambient temperature, *e.g.* $[Cl]^-$, $[NO_3]^-$, $[OAc]^-$, and $[CF_3COO]^-$.[62,63] The aqueous solubilities of hydrophobic ionic liquids can be fine-tuned by the cations structures. For instance, a longer alkyl chain usually makes the ionic liquid less soluble in water.[56,62] When alkyl chains on 1-position of cations are shorter than 6 carbon atoms, the tendency of aqueous solublities followed: $[C_n mIm][Tf_2N] > [C_n mPyr][Tf_2N] > [C_n mPy][Tf_2N]$.[64] When the chains attain 8 carbons, the solubilities of $[C_8 mIm][Tf_2N]$, $[C_8 mPy][Tf_2N]$ and $[C_8 mPyr][Tf_2N]$ are almost the same, between 1 and 3 $mmol.L^{-1}$. Introduction of an additional methyl group at the C3 position of pyridinium ring reduces the solubility, while the presence of hydroxyl or ester groups in the side alkyl chain on cations increases the solubilities.[58]

2.3.3 Octanol-water partition coefficient

The 1-octanol-water partition coefficient (K_{OW}) is an important factor determining movement and distribution of chemicals in the environment.[65] It is defined as the ratio of the equilibrium concentrations of the test substance in 1-octanol saturated with water (C_O) and water saturated with 1-octanol (C_W):[66]

$$K_{OW} = C_O / C_W \quad (1)$$

1-Octanol is an organic solvent that contains both a hydrophobic hydrocarbon chain and a hydrophilic end group and is only partially miscible with water. Hence it provides a more realistic representation of the physico-chemical environment in living tissue than a purely nonpolar solvent, such as hexane.[28] K_{OW} is commonly used as an indicator of the lipophilicity of a chemical, based on the distribution of a chemical between a lipid (represented by octanol) and water in a two-phase environment. K_{OW} plays a significant role in partitioning into lipid bilayers of biomembranes[67] (Figure 2.5 left) and toxic effect[68,69] of chemicals.

As a measurable property, chemical's K_{OW} can be used to estimate many of the more difficult to measure properties that control chemical movement and fate, such as soil sorption coefficient and bioconcentration factor (BCF, Figure 2.5 right).[28] Furthermore, K_{OW} is widely used as a parameter for quantitative structure–activity relationship (QSAR) and plays an important role in the early stages of an environmental risk assessment for a chemical.[68]

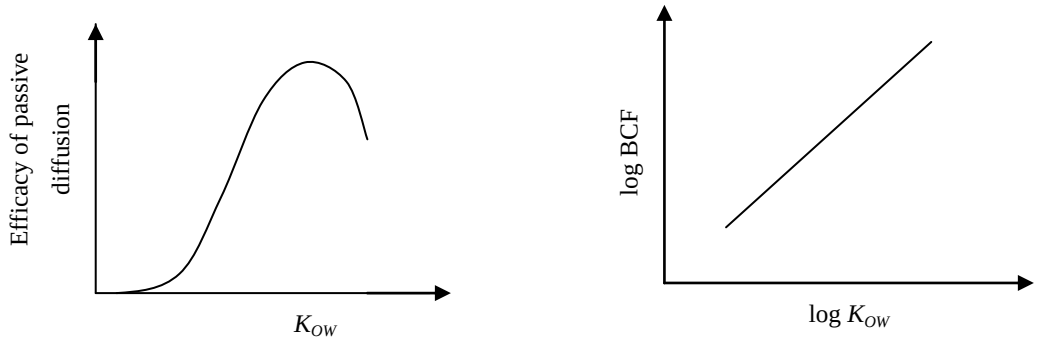


Figure 2.5 Correlation of K_{OW} with other properties for xenobiotics (left) Efficacy of passive diffusion across a biological membrane; (right) Bioconcentration factors (according to Walker *et al.*[27]).

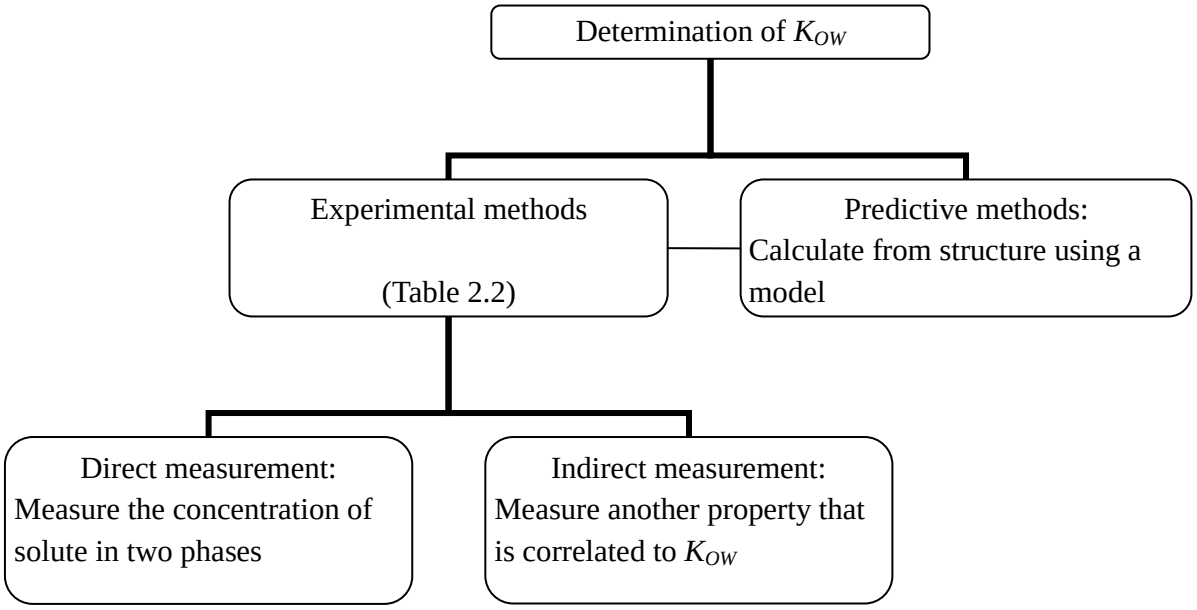
The K_{OW} values of chemicals with different structures can vary significantly. K_{OW} measures the hydrophobicity of the chemical species. K_{OW} of some common compounds are listed in Table 2.1. Chemicals with $K_{OW} \geq 10^4$ are considered as hydrophobic which is possible to cause chronic toxic effect to the organisms.

Table 2.1. K_{OW} of OECD recommended reference compounds.[70]

compounds	K_{OW}	[a]Class	compounds	K_{OW}	Class
ethanol	0.57	n	Toluene	501	n
acetone	0.50	n	1-Naphthol	501	A
2-Butanone	2.00	n	Allyl phenyl ether	794	n
Acetanilide	10.0	n	Bromobenzene	1000	n
Benzyl alcohol	12.6	n	Ethylbenzene	1585	n
Phenol	31.6	A	4-Phenylphenol	1585	A
Benzene	126	n	2,6-Diphenylpyridine	79433	n
4-Chlorophenol	251	A	Triphenylamine	501187	n
Atrazine	398	B	DDT	1584893	n

[a]A: acidic compounds; B: basic compounds; n: neutral compounds

A large number of methods for determination of K_{OW} is proposed in literature, which can be classified into two groups – experimental and predictive methods (Scheme 2.1).



Scheme 2.1. Methods for determination of $K_{OW.}$

A. Experimental methods

In direct methods, a two-phase system is equilibrated and the concentration of solute in one or both phase(s) is measured. In indirect methods, another property of a training set of solutes with known K_{OW} values is measured, and then a property-K_{OW} model is

constructed. The K_{OW} is then obtained by measuring the appropriate property. The adopted properties in indirect methods include volumes occupied by the stationary and mobile phase, retardation factor and retention time from High-Performance Liquid Chromatography (HPLC) with/without solid support, activity coefficients, aqueous solubility and so on.[27]

The importance of the correct measurement of this property is undefined by the Organization for Economic Cooperation and Development (OECD) which proposes four guidelines for measuring K_{OW}: Shake-flask method (OECD 107), slow-stirring method (OECD 123), High-Performance Liquid Chromatography method (OECD 117) and pH-metric method (OECD 122). These standard methods make the experimental data more reliable reducing the discrepancy values determined by different research groups.

Table 2.2. Frequently used experimental methods for determining K_{OW}.

Shake-flask	The flask containing the mixture system is shaken for enough time to make the substance to become distributed between the two phases
Slow-stirring method	The mixture system is stirred slowly for an extended period to attain the equilibrium of distribution
Sit-flask method	equivalent to the shake-flask method without shaking
HPLC approach	substance partition between a non-polar liquid organic phase and a polar aqueous phase in a column.
pH-metric method	An aqueous solution of the sample (weak acid) is titrated with 0.5 M KOH to some appropriately high pH. Octanol is then added and the mixture is titrated with 0.5 M HCl back to the starting pH. The two part assay yields two constants. A large difference between the two constants indicates a large K_{OW} value

The applicability, advantages and weaknesses of some frequently used measurement methods are studied in literature.[28,66,69,71] Here, we simply cite the comparison made by Kah *et al*. (Table 2.3).[71]

Table 2.3. Comparison of methods to determine K_{OW} (according to Kah *et al.*[71]).

Method	Advantage / Drawback	Not applicable if
Shake-flask	Most realistic method, simple, reliable	logK_{ow} > 6; the tested substance dissociates (K_{ow} depends on concentration)
	Time consuming, formation of octanol emulsions in water	
Slow stirring method	Avoid the formation of emulsions, reliable	The tested substance dissociates (K_{ow} depends on concentration)
	Time consuming	
Classical HPLC approach	Rapid and cheap, does not require chemical quantification	Very low K_{ow} values
	Poor reproducibility; pH range limited to 2-7.5	
pH metric method (potentiometric method)	Rapid	
	Expensive equipment required Insoluble compound	

In general, the direct experimental methods are reliable, but rather time-consuming, especially for the compounds having high K_{OW} values because they need a long time to equilibrate. The measurement is easily disturbed by many factors, for example, the impurities in the used chemicals, the formation of microdroplets of octanol in water, the waiting time for equilibrium, the association and dissociation of solutes in different phases *etc*. Indirect methods are usually quick and cheap, but less accurate and reliable. They need more equipments and data support. As a consequence, it is frequent to see that the K_{OW} values for the same compound in different literature sources are rather disperse, even when using the same methods.

B. Predictive methods

A number of cost effective and rapid models, quantitative structure-property relationships (QSPR) are used to predict or estimate the properties of compounds, even including the chemicals not yet synthesized or those that cannot be examined experimentally due to their extremely hazardous nature. QSPR can be classified as being fundamental, empirical, or semi-empirical, which is limited by the unavailability of the experimental properties for the majority of chemicals of environmental concern.[72] The

satisfactory mathematical models should be accurate, reliable and no more complex than necessary.

QSPR use fundamental relationships, fragment contributions, or topological indices, to account for the relationship between the molar properties and the molecular structure of chemicals.[28] Estimation of K_{OW} values using QSPR models without any input of experimental data can be applied to a wide range of chemicals and is normally reliable for neutral compounds.

With the help of powerful digital computers, there are at least 20 software packages for estimating K_{OW} of a chemical by theoretical calculation. The softwares "AlogP" and "XlogP" from Virtual Computation Chemistry Laboratory (www.vcclab.org), "KowWin 1.67 (EPI Suite™ package)" from US EPA/ Syracuse Research Corp. (www.epa.gov), and "SPARC" form University of Georgia (http://ibmlc2.chem.uga.edu/sparc/) are freely available.

Since the 1970s, various models for predicting K_{OW} have been built and reviewed.[28,71,73,74] In these references, the models are categorized according to different aspects or detail levels (Table 2.4).

Katritzky *et al*.[75] have divided the descriptors used in QSPR into 8 groups: (1) Constitutional (2) Topological (3) Geometrical (4) Electrostatic (5) Charged partial surface area (CPSA) (6) Quantum-chemical (7) MO-related (8) Thermodynamic. In this study, we categorize the predictive models for K_{OW} into: (1) Constitutional (2) Topological (3) Geometrical or (4) Combination methods.

The electrostatic descriptors reflect characteristics of the charge distribution of the molecule.

Table 2.4. Description of some models in the literature.

Reference	Categories
Leo[73]	1. By substituents 2. By Fragments using C_M 3. By atomic contributions and/or surface area 4. By molecular properties 5. By solvatochromic parameters
Mannhold and Waterbeemd[74]	1. Substructure approaches 2. Whole molecule approaches
Ruelle[76], and Baum[77]	1. Atomistic method; 2. Fragment or group contribution methods; 3. 'Whole molecule' approaches 4. Other methods
Cheng *et al.*[78]	1. Additive methods; 2. Property-based methods
Padmanabhan *et al.*[79]	1. Substituent constant approach 2. By thermodynamically oriented theories 3. Connectivity indexes and characteristic root index 4. Molecular volume and/or total surface area 5. Molecular refraction 6. By quantum chemistry

(1) Constitutional methods

This kind of methods uses the concept of "constitutional descriptors", which are parameters related to the number of atoms and bonds in each molecule. Several kinds of parameters can be used as the atom fragments or groups or molecular structures.

These methods are based on the assumption that K_{OW} is an additive property. The molecular structure is generally divided into fragments (atoms or larger functional groups) and the values for each group are summed (sometimes with correction factors) to estimate K_{OW}. Mannhold and van de Waterbeemd[74] have overviewed the details, the advantages and drawbacks of fragmental approaches. This method is applicable to a wide range of chemicals (e.g. highly lipophilic classes of compounds) and, as in the case of other empirical methods, can be greatly improved when experimental K_{OW} values of similar molecules are known.

(2) Topological methods

Topological descriptors describe the atomic connectivity in the molecule, including valence and non-valence molecular connectivity indices calculated from the hydrogen-suppressed formula of the molecule, and encode information about the whole molecule in terms of the size, shape, symmetry, composition, atom distribution and degree of branching.[80,81]

These methods have been shown to outperform traditional empirical, ones especially for closely related compounds being less suitable for compounds containing heteroatoms.[82]

(3) Geometrical methods

The geometrical descriptors describe the size of the molecule and require 3D-coordinates of the atoms in a given molecule. The mostly used descriptor for K_{OW} is the molar volume (V_m). Wang *et al.*[83] have correlated K_{OW} to V_m by regression analysis for 20 chemical classes, which included 317 organic chemicals and a good correlation was found. Ruelle[76] extended the non-ergodic thermodynamic treatment of the mobile order and disorder (MOD) theory in H-bonded liquids to the prediction of K_{OW}, which is also available in any mutually saturated two-phase system made up of two largely immiscible solvents. Constructed from the various free energy contributions encoded in the distribution process, the prediction model allowing the prediction of logK_{OW} from a linear correlation of V_m is finally proved.

(4) Combination methods

Some models have employed more than one kind of descriptors. We classified them as combination models in this work.

For example, topological and quantum-chemical parameters are adopted together to build linear regression models.[82] The quantum chemical descriptors offer information about binding and formation energies, partial atom charge dipole moment, and molecular orbital energy levels.[84]

The MO-related descriptors, highest occupied molecular orbital energies (E_{HOMO}) and lowest unoccupied molecular orbital energies (E_{LUMO}) are often combined with other

parameter for the constructions of models.[79,85]

C. Studies on K_{OW} of ionic liquids

From the point of view of consistency of interpretation, it is preferable to define the partition coefficient as referring to the same molecular species in both phases, which are called "true/intrinsic partition coefficient", or "partition constant".[67,69,86] The true K_{OW} of an ionic liquid can be viewed as the ratio of the concentration of the ions, C^+ and A^-, in the two phases.

The studies on K_{OW} of ionic liquids are extremely limited and the most commonly studied ionic liquids are those based on imidazolium cations. Ropel *et al.*[69] have used a slow-stirring method to test 12 imidazolium-based ionic liquids. Lee and Lee[67] have used shake-flask method to test two [C_4mIm] based ionic liquids. Choua *et al.*[87] have used shake-flask method (which is not the OECD standard method) to test [$C_{2\text{ or }4}$mIm][PF_6]. In these studies using direct methods, the experimental values varied with the concentration of ionic liquids, except [C_4mIm] based ionic liquids with non-[Tf_2N] anions in the study of Ropel *et al.*[69] (Table 2.5). In the studies of Ropel *et al.*[69] and Lee and Lee[67], the concentrations determined by UV-vis detector correspond actually to all the species containing an imidazolium ring. So the K_{OW} values they obtain were the ratio of both cations and ion pairs in two phases, which they call apparent partition coefficient.

Domanska *et al.*[88] have calculated the K_{OW} of [C_nmIm][Cl] by measuring the solubility of ionic liquids in water and in 1-octanol by different methods, which do not really correspond to the definition of the octanol-water partition coefficient (Table 2.5). Chapeaux *et al.*[58] used the measured aqueous solubility data and made use of the nonrandom two liquid (NRTL) and electrolyte nonrandom two liquid (eNRTL) excess Gibbs energy models for training data to predict K_{OW} of some imidazolium, pyridinium and quaternary ammonium ionic liquids (Table 2.6).

Stepnowski and Storoniak[89] have calculated K_{OW} of [$C_{3\text{-}6}$mIm][BF_4] from their structures, where the K_{OW} value of [C_4mIm][BF_4] is 12 times larger than the experimental data of Ropel *et al.*[69] (0.036 to 0.003).

Table 2.5. K_{OW} of [C_nmIm] ionic liquids between 20 ℃ - 30 ℃ in reported literature.

Ionic liquids	K_{OW}	Ionic liquids	K_{OW}
[C_2mIm][Tf_2N]	0.09-0.11[69]	[C_3mIm][BF_4]	0.0182[89]
[C_4mIm][Tf_2N]	0.11-0.62[69] 0.02-3.16[67]	[C_4mIm][BF_4]	0.0363[89]; 0.0030[69]
[C_6mIm][Tf_2N]	1.42-1.66[69]	[C_5mIm][BF_4]	0.0813[89]
[C_8mIm][Tf_2N]	6.3-11.1[69]	[C_6mIm][BF_4]	0.1950[89]
[C_4mIm][Cl]	0.0040[69] 0.48[88]	[C_2mIm][PF_6]	0.015[87]
[C_8mIm][Cl]	0.54[88]	[C_4mIm][PF_6]	0.02[87]; 0.022[69];0.004-0.1[67]
[C_{10}mIm][Cl]	0.52[88]	[C_4mIm][NO_3]	0.0038[69]
[C_{12}mIm][Cl]	0.73[88]	[C_4mIm][Br]	0.0033[69]
[C_2mIm][$B(CN)_4$]	0.169[58]		

[69]slow-stirring method; [67,87]shake-flask method; [88, 58]indirect method; [89]calculation method

Table 2.6. K_{OW} of pyridinium and ammonium-based ionic liquids.[58]

Ionic liquids	NRTL	eNRTL	Ionic liquids	NRTL	eNRTL
[C_4mPy][Tf_2N]	1.62	1.4	[C_8mPy][Tf_2N]	23.8	7.22
[C_6Py][Tf_2N]	5.88	2.9	[C_6(NCC)Py][Tf_2N]	23.8	11.3
[C_6mPy][Tf_2N]	11.8	4.71	[C_6m(NCC)Py][Tf_2N]	23.8	15.2
[C_6mmPy][Tf_2N]	23.8	9.21	[N_{1112OH}][Tf_2N]	0.268	
[C_6emmPy][Tf_2N]	7.89	5.09			

In a word, the highest K_{OW} value obtained from various methods did not exceed 25, which is far from the values that can conduct to high toxicology effect. Compared to OECD recommended reference compounds, K_{OW} values of ionic liquids are amongst the lowest ones. Furthermore, the discrepancy of the values from various methods is not big enough to compromise an evaluation of the environmental impact for these compounds.

The influence of molecular structures on K_{OW} can be observed. Pyridinium ionic liquids have larger K_{OW} than imidazolium ionic liquids. For the ionic liquids with a same anion, K_{OW} increases as the alkyl side chain on the cation increases. K_{OW} of ionic liquids with $[Tf_2N]^-$ are larger than those of ionic liquids with other anions, *eg.* $[Br]^-$, $[BF_4]^-$, $[NO_3]^-$, $[Cl]^-$ and $[PF_6]^-$, while those of ionic liquids with $[Br]^-$, $[NO_3]^-$ or $[Cl]^-$ are extremely low. The dimethylamino group can increase K_{OW} of ionic liquids.

We can predict that, in a general way, these ionic liquids show a weak affinity of

accumulation in the environment and organism. They have low efficacy of passive diffusion across a biological membrane, low possibility of being stored in lipophilic sites. For aquatic organisms, low biocentration factors are associated with low K_{OW} values. If these ionic liquids enter terrestrial ecosystems, they tend to dissolve in water and are adsorbed to soil colloids only to a limited degree. As consequence, they have low possibility of persistently to bioaccumulate along terrestrial food chains.

2.3.4 Transport properties

A. Viscosity

Viscosity is a transport property of a fluid that offers resistance to flow due to internal friction. Viscosity provides useful information about the structure and molecular interactions present in ionic liquid media, as they determine the way the ions move in the liquid state.[90] Viscosity is the substance constant indicating the magnitude of the fluidity of a fluid. From a practical point of view, viscosity is a key parameter linked to the pump costs, mass transfer and power requirements for stirring in an engineering process.[43,,91] With the increasing number of applications of ionic liquids, knowing the viscosity becomes necessary and useful.

The viscosities of ionic liquids at ambient conditions generally range from 22 to 40000 cP, which are two to three orders of magnitude greater than those for conventional organic solvents.[4,92]

Due to the difficulty of measurement, viscosity of ionic liquids is less investigated than density, and most of the studies focus on imidazolium-based ionic liquids. The viscosity can also be considerably affected by the presence of water or other impurities[42,43], a large discrepancy being found in the literature.

Viscosity is influenced by different factors related to the molecular structure of the ionic liquid (van der Waals interaction, H-bonding, geometric shape, ionic size, charge location or polarizability[93,94]). Contrary to density, viscosities of ionic liquids increase with the alkyl side chains of the cations,[43,44] or of the anion, or of both.[95] This behavior confirms that, although the high-electrostatic interactions between ions determine the characteristic peculiar physico-chemical properties of ionic liquids, dispersive, van der

Waals-type forces also play an important role, namely determining the way the viscosity changes with the size of the alkyl-side chain.[96] A more complex behavior than the one found for the molar volumes is observed and so it was not possible to implement group contribution prediction schemes in this case. A general order of viscosities for the ionic liquids formed with different cations and an identical anion has been proposed: $[C_4mIm]^+ < [C_4Py]^+ < [C_4mPyr]^+ < [N_{4111}]^+$.[97]

The temperature dependence of the viscosity of ionic liquids is commonly fitted to the empirical models, Vogel-Fulcher-Tammann ($\eta = \eta_0 T^{-1/2} \exp[B / (T - T_0)]$), where η_0 is a pre-exponential constant proportional to the viscosity coefficient, and it is assumed as the ideal viscosity at the infinitely high temperature. *B* is the pseudo-activation energy for viscous behavior, and T_0 is the ideal glass transition temperature or Litovitz equations, which are extensions of the Arrhenius equation ($\eta = \eta_\infty \exp(E_\eta/RT)$, where E_η is activation energy for viscous flow and η_∞ is the viscosity at infinite temperature, which describes the temperature dependence for non-associating electrolytes.[98]

B. Diffusion coefficient in water

Diffusion is the transport of mass caused by random molecular motion in the medium. Diffusion behavior may help obtaining an understanding of interactions with these systems. Intradiffusion (self-diffusion) is the interchange of labeled or unlabeled species under conditions of uniform chemical composition. Interdiffusion (mutual diffusion), in contrast, occurs in the presence of a binary or multicomponent system. It smoothes out gradients in chemical composition by allowing random thermal motions to interchange different chemical substances.[99] One of the fundamental laws for describing diffusion, Fick's Law of diffusion, uses the diffusion coefficient in the mathematical model:

$$J = -D_{AB} \frac{\partial C}{\partial x} \qquad (2)$$

where, *J* measures the amount of substance that will flow through a small area during a small time interval; D_{AB} is the diffusion coefficient or the diffusivity, *C* is the concentration of substance; *x* is the position (length). *D* depends on the temperature, on the

viscosity of the fluid, on the size of the particles and the interaction between the particles and the solvent.[100]

Intradiffusion coefficients provide fundamental information about solvation and the structure and dynamics of solutions,[101] and there are more studies on self-diffusion of ionic liquids than on interdiffusion, however, it is less important for environment.

Interdiffusion coefficients are important in different fields of application. For example, in chemistry, they can determine the rate of chemical reactions; in physiology, they can control several processes like the rate of digestion; in chemical industry, diffusion can govern the rates of liquid-liquid extractions. Diffusion coefficient of chemicals in water is also a very important physical property to understand their transport in environmental compartments, both in water and in the soil.[102]

Diffusion coefficients of ionic liquids are often measured using the Taylor dispersion technique, in an apparatus similar to the one reported by Cussler.[102] A concentration pulse of solute was injected into a laminar flow of solvent, giving rise to a Gaussian concentration peak after a known length of tube (or elution time). The experimental setup used here is shown in Figure 2.6.[103]

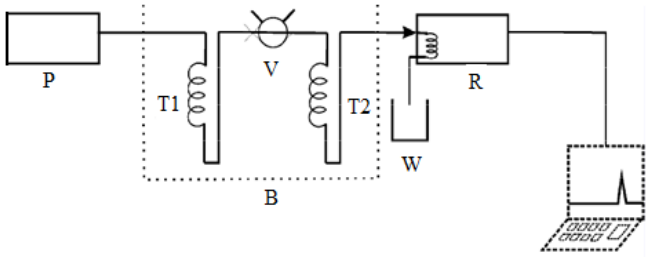


Figure 2.6. Taylor dispersion-diffusion measurement apparatus: P, pump; T1, preheating coil; V, six-way valve; T2, diffusion tube; B, thermostatic bath; R, conductivity and refractive index detectors; W, waste (according to Sarraute *et al*.[103]).

In dilute aqueous solutions, the diffusion coefficients of most ions are similar and have values that, at room temperature, are in the range of 0.6×10^{-9} to 2×10^{-9} $m^2.s^{-1}$. For biological molecules, the diffusion coefficients normally range from 10^{-11} to 10^{-10} $m^2.s^{-1}$.[100] The diffusion coefficient of ionic liquids in water is rarely reported, while only [C_nmIm]

ionic liquids with different anions have been studied.[103,104,105] The experimental diffusion coefficients values for ionic liquids in water at infinite dilution increase with temperature and vary from 0.9 to 1.5 $\times 10^{-9}$ $m^2.s^{-1}$ at 303 K. The coefficients of ionic liquids are closely related to their molar mass.

Simple heuristic relations between the molecular weight of the solute and the diffusivities of ionic liquids in water fail to predict the trends found experimentally.[103] The variation of the diffusivities with temperature is not correctly predicted by the Stokes-Einstein equation ($D_{AB} = \frac{k_B T}{6\pi\eta_A r_B}$, where k_B is the Bolzmann constant, T is the temperature, η_A is the viscosity of the solvent; and r_B is the effective hydrodynamic radius of the solute), a more complete correlation, for example, the Wilke–Chang model ($D_{AB} = 7.4\times10^{-12} \frac{(\psi M_A)^{0.5} T}{\eta_A V_B^{0.6}}$, where ψ is the association parameter for the solvent (water = 2.26[104]), M_A are the molar mass of solvent, V_B is the molar volume of the solute), being in general, necessary.

3. Bibliographic Review

Due to the interesting and tunable physico-chemical properties of ionic liquids, the development of their applications at industrial scale is growing in a great extent. Nevertheless, the deficiency of information or data on their environmental fate and impact in the environment could limit their large use due to several new and more strict regulations and directives (European Community Regulation on chemicals, REACH for example). Therefore studies on toxicity assays on various biological models, in particular aquatic ones, getting from the microorganism to the upper mammals, have provided extensive data sets and brought some general trends concerning the structure-activity relationship.

Several reviews have been recently published on the subject.[2,106,107,108] In this bibliographic part, an overview of the published studies is presented in two parts: 1. the toxicity studies of ionic liquids; 2. the biodegradability and biodegradation pathways of imidazolium, pyridinium and ammonium-based ionic liquids. This review focuses on the influence of the ionic liquid structures on their biological effect. The details of the experimental methods have not always been included.

3.1 Toxicity studies of the ionic liquids

The evaluation of toxicity is generally performed by exposing a target organism or systems of different levels of biological complexity to the tested compound at different concentrations for a specified duration, and then establishing the relationship between dose and adverse effects. The data obtained correspond to acute toxicity. Fewer studies deal with the damage effect due to the repetition of a low dose application on a long-term period (chronic toxicity).

The difficulty in such studies is the choice of a pertinent biological model and the number of individuals to test: they typically have different levels of response to the same dose of toxin. Therefore it is important to carry out tests with several species of organisms[109] or across all levels of biological organisms from the molecular to whole communities and ecosystems.[110] In general, very high precision cannot be achieved because of the variation which exists naturally within populations of organisms.[109] So in

this review, the deviation or uncertainty of values reported in literatures are not cited.

Moreover, in the environment, the presence of several chemicals can have an effect on the organism studied and lead to a modified answer of this organism toward the toxin studied. The toxicity of chemical mixtures is more difficult to determine than that of a pure chemical, because each component displays its own toxicity, and components may interact to produce enhanced or diminished effects. That is the reason why most of the toxicity studies are carried out in the laboratory and give potential effect of the chemical studied.

At present, more than one hundred publications concerning the toxicity of ionic liquids have been published. Most of them have been carried out with aquatic biological models for practical reasons such as ease of testing[109] but also because this compartment is the most probable receptor of ionic liquids. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) has put forward a system for classifying chemical substances based on the hazards they present to the aquatic organisms.[111] These criteria are summarized in Table 3.1.

Table 3.1. Hazard categories to the aquatic environment.[111]

	96 hr [a]LC_{50} (for fish)	48 hr [b]EC_{50} (for crustacean)	72 or 96 hr LC_{50} (for algae or other aquatic plants)
Category: Acute 1	≤ 1 mg.L^{-1}	≤ 1 mg L^{-1}	≤ 1 mg L^{-1}
Category: Acute 2	> 1 - ≤ 10 mg L^{-1}	> 1 - ≤ 10 mg L^{-1}	> 1 - ≤ 10 mg L^{-1}
Category: Acute 3	> 10 - ≤ 100 mg L^{-1}	> 10 - ≤ 100 mg L^{-1}	> 10 - ≤ 100 mg L^{-1}
Category: Chronic 1	≤ 1 mg L^{-1}		≤ 1 mg L^{-1}
	and the substance is not rapidly degradable and/or $K_{OW} \geq 10^4$		
Category: Chronic 2	> 1 - ≤ 10 mg L^{-1}	> 1 - ≤ 10 mg L^{-1}	> 1 - ≤ 10 mg L^{-1}
	and the substance is not rapidly degradable and/or $K_{OW} \geq 10^4$		
Category: Chronic 3	> 10 - ≤ 100 mg L^{-1}	> 10 - ≤ 100 mg L^{-1}	> 10 - ≤ 100 mg L^{-1}
Category: Chronic 4	Poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility		

[a]LC = lethal concentration: the dose causing death of median exposed organisms; [b]EC_{50} = half maximal effective concentration

At the early stage of studying the toxicity of ionic liquids, Jastorff and coworkers

estimated the environmental risk of ionic liquids, using a combination of structure–activity relationships (SAR), toxicological and ecotoxicological tests and modeling.[112] Their strategy presents a suitable methodology for ionic liquid toxicological investigations. Because the estimation is based on the data of structural elements forming ionic liquid structures, the uncertainty of the risk evaluation is rather high. Hence the requirement for first-hand experimental data is essential. For ionic liquids, the effects of the cation and of the anion as a function of their chemical structure on the toxicity towards different biological test systems have been reported.

Several reviews on this subject have been recently published.[106,107] Some toxicity data, expressed as EC_{50} of ionic liquids, are listed at the end of this section. The non-functionalized ionic liquids are presented in Table 3.13 while the functionalized ionic liquids are in Table 3.14. The studies on toxicity of ionic liquids will be presented by the effect at different complexity levels including microorganisms, enzymes, algae and other plants, crustacean, mammalian cells and animals.

3.1.1 Toxicity on microorganisms

Microorganisms play important roles in all ecosystems and are vital to the environment due to their degradative capacities and involvement in the biogeochemical cycles of Earth. Since bacteria have short generation times, are sensitive to the environment, and easy to conserve, they usually serve as an ideal starting point for ionic liquid toxicity estimations.[106] Two distinct types of bacteria based on the structural, chemical and physical property differences of their bacterial cell walls are defined: Gram-positive bacteria, that will retain the crystal violet dye of Gram, possess a thick cell wall whereas Gram-negative bacteria have a relatively thin cell wall containing an outer membrane.[106]

A. Bioassay Microtox®

The Microtox® test is a standardized, rapid, sensitive, reproducible, ecologically relevant and cost effective test of acute toxicity.[113] It is based on the bioluminescent activity of the marine bacterium *Vibrio fischeri* (formerly *Photobacterium phosphoreum*,

Gram-negative). The procedure consists in the determination of the pollutant concentration that inhibits 50% of the luminescence produced by this bacterium after 15 min of exposure (EC_{50}).

In most of the published studies, the authors have compared the effects of different ionic liquids and of traditional organic solvents. The EC_{50} values obtained on *V. fischeri* in these different works are often quite different due to different protocols or to different answers of the living microorganism but the trends remain the same (Table 3.2).

Table 3.2. Examples of the EC_{50} values obtained with the acute toxicity test of Microtox® for common organic solvents and some ionic liquids.

Compound	log EC_{50} ($\mu mol.L^{-1}$)[12]	log EC_{50} ($\mu mol.L^{-1}$)[114]	log EC_{50} ($mmol.L^{-1}$) [115]
Methanol	6.51	5.55-7.00	3.50
Acetone	5.17	5.35-5.70	2.52
Benzene	2.03	1.41-3.12	0.14
Phenol	-	2.35	- 0.49
Dichloromethane	4.57	4.05-4.53	-
Chloroform	3.08	3.55-4.32	-
[C_4mIm][Cl]	1.85	3.39	0.71
[C_6mIm][Cl]	1.09	2.11	- 1.58 [Br]
[C_8mIm][Cl]	0.54	0.94	- 2.37 [Br]
[C_4mPy][Br]	-	-	0.40
[C_6mPy][Br]	-	-	- 0.94
[C_8mPy][Br]	-	-	- 2.21

Many of the ionic liquids tested are more toxic than the common high-volume organic solvents. The longer alkyl chain of the alkylsubstituent results in a remarkable increase of the acute toxicity.[12,113,114,115] Romero *et al.*[114] have found a linear relationship between the alkyl chain length (C_1 to C_8) on the cation and the toxicity. Conversely, the influence of the anion is minimal[114,116,117,118,119], except in the case of $[Tf_2N]^-$ that was found to increase toxicity.[120] The family of the cation has also an influence on the toxicity getting from less toxic to more toxic as ammonium < pyridinium < imidazolium.[115] In addition, toxicity is expected to decrease with ring methylation as well as with an increase in the number of

negatively charged atoms on the cation.[115] The introduction of one oxygen atom in the side chain of imidazolium cation increases the polarity and reduces greatly the toxicity but further addition of ethoxy moieties causes an increase of the toxic effect with this test.[117]

B. Antimicrobial activities

Many research groups report anti-microbial activities of ionic liquids on a variety of microorganisms (yeast, fungi and bacteria). Two parameters of toxicity can be monitored: the Minimum Inhibition Concentration (MIC) that inhibits the microbial growth and the Minimum Bactericidal Concentration (MBC) corresponding to the lowest concentration of chemical required to kill the bacterium tested.

Many research works showed a clear trend towards a stronger toxic effect with the increase in length of the alkyl side chain of imidazolium and pyridinium-based ionic liquids, whatever the microorganisms tested.[114,115,116,121,122,123,124] This effect was reduced when the alkyl chain reaches $C \geq 12$, probably due to steric effect[125] although $[C_{14}mIm]^+$ and $[C_{16}mIm]^+$ were described as biocides by Morrissey *et al.*[19] In these cases, the toxicity is correlated with the lipophilicity of the cation, suggesting strong interaction with the surface of the microbial cells. When the chain gets longer, these interactions may be limited and the solubility in water decreased as well. This class of ionic liquids has been supposed to act as detergent and probably lead to cell wall damage or even disruption.[117]

The toxicity is clearly reduced (partially by six to seven orders of magnitude) when short-functionalized side chains replaced non-polar alkyl chains.[126] A significant reduction in toxicity for the ionic liquids containing at least one oxygen atom (ester, ether or poly ether) in the side chain was observed.[19,127] Incorporation of a methyl group in the 1-position[19] or a 2-hydroxyethyl group in 3-position[128] has no significant effect on toxicity whereas the introduction of an amide function decreases toxicity.[19]

The anion seems to have not a significant effect on antimicrobial activity when associated with imidazolium cations.[12,19,116,121,123,124,125] Only the L-lactate salts were found to be more toxic than DL-lactate salts.[123] Conversely the anion associated with alkyltrihexylphosphonium has been shown to have a great effect on antimicrobial properties: halide salts are strongly effective against cocci and rods and present activities

comparable to a standard commercial biocide (benzalkonium chloride). Exchange of the halide to other anions resulted in a complete loss of the antimicrobial activity.[129] The more symmetrical anion is found to be less adsorbed at the surface of cell membrane.[129] In this case, both cation structure and the type of anion had effects on the biological activity.

For all these studies, it is often difficult to compare the results as the conditions of cultivation and growth (media, cell density), the starting ionic liquid concentration, the procedure of dilution are different. Moreover, the tolerance by a microorganism is dependent on the genus, the species and the strain of microorganism.[121,129] Some studies have shown that Gram-negative strains are distinctly more tolerant than Gram-positive ones, that could be explained by the fact that they have a more chemically complex cell wall and an additional outer membrane.[125] The Gram-negative bacteria are sometimes more susceptible than Gram-positive bacteria to certain [(OC_n)Im][DL-lactates] ionic liquids.[123] Others found differences between the bacterial strains tested but not related to the cell walls characteristics. For example, Docherty and Kulpa[116] found the greatest inhibition for *Bacillus subtilis* (Gram-positive) towards $[C_8mIm]^+$ whereas almost no effect was observed on *Staphylococcus aureus* also Gram-positive. Therefore other physiological characteristics should be taken into account. Filamentous fungi have also been included in these tests. Fungi tolerate higher concentrations of ionic liquids than bacteria: the imidazolium cations were the most toxic, followed by the groups of pyridinium, pyrrolidinium, morpholinium; the cholinium were the less toxic.[130] The cholinium alkanoates were less toxic than their corresponding sodium salts.[131] $[NH_{11(2OH)}][Ac]$ and $[N_{111(2OH)}][Me_2PO_4]$ even increased the growth rate of the bacteria *Clostridium sporogenes*.

In conclusion from all the studies published (Table 3.3.), the structure of the cation is the main factor of the toxicity of ionic liquids to microorganisms, in particular the length of the alkyl side chain. The incorporation of oxygen into this chain is crucial to reduce or even suppress toxicity. Anions exert practically no effect on anti-microbial activity in the case of the series of imidazolium-based ionic liquids but have a key role in phosphonium-based ionic liquids. The real biological effect of ionic liquids in function of the microorganism (cell wall characteristics, bacteria versus fungi) is not completely elucidated.

Table 3.3. Synthesis of published studies on ionic liquids toxicity toward microorganisms.

Authors	Tested ionic liquids type(s)	Target(s)	Indicator/Method
Garcia *et al.*[12]	$[C_nmIm]^+$ + $[PF_6]^-$, $[BF_4]^-$, $[Cl]^-$, $[Br]^-$ (n = 4,6,8)	*V. fischeri*	EC_{50}
Morrissey *et al.*[19]	Im (ester, ether)	7 bacteria	MIC
Ranke *et al.*[113]	$[C_nmIm]^+$ + $[PF_6]^-$, $[BF_4]^-$, $[Cl]^-$, $[Br]^-$ (n = 3-10)	*V. fischeri*	EC_{50}
Romero *et al.*[114]	$[C_nmIm]^+$+ $[PF_6]^-$, $[Cl]^-$, $[C_{1\ or\ 2}SO_4]^-$ (n = 1-8)	*V. fischeri*	EC_{50}
Couling *et al.*[115]	N, Py, Im, triazolium, tetrazolium	*V. fischeri*	EC_{50}
Docherty and Kulpa[116]	$[C_nmIm][Br]$, $[C_nPy][Br]$ (n = 4,6,8)	*V. fischeri* + 5 microbial strains	EC_{50}, growth rate
Samori *et al.*[117]	$[C_4mIm]^+$, $[(C_2O)_nC_1mIm]^+$+ $[BF_4]^-$, $[N(CN)_2]^-$ (n = 1-4)	*V. fischeri*	EC_{50}
Luis *et al.*[118]	$[C_nmIm]^+$+ $[Cl]^-$, $[Br]^-$, $[PF_6]^-$, $[BF_4]^-$, $[C_{n'}SO_4]^-$, $[N(CN)_2]^-$ (n = 2-10, n' = 1,2) mPy, $[C_{n''}mPy]^+$, $[C_4Py]^+$, $[C_4mmPy]^+$ + $[Cl]^-$, $[Br]^-$, $[N(CN)_2]^-$ (n'' = 4,6,8)	*V. fischeri*	EC_{50} , model
Matzke *et al.*[119]	$[C_nmIm]^+$ + $[(CF_3)_2N_2]^-$, $[BF_4]^-$, $[Cl]^-$, $[C_8SO_4]^-$, $[Tf_2N]^-$, bis(1,2-benzenediolato)borate (n = 2,4,8)	*V. fischeri*	EC_{50}
Stolte *et al.*[120]	Py, Im, Mor, Pyr, N, (ether, hydroxyl and nitrile)	*V. fischeri*	EC_{50}
Samori *et al.*[132]	$[C_4mIm][BF_4]$	*V. fischeri*	EC_{50}
Ventura *et al.*[133]	$[C_3mIm][Tf_2N]$	*V. fischeri*	EC_{50}
Zhang *et al.*[134]	$[C_4mIm][PF_6]$, $[C_2Py][BF_4]$, $[C_2Py][CF_3COO]$	*Pseudomonas fluorescens*	growth rate
Matsumoto *et al*[121]	$[C_nmIm][PF_6]$ (n =4,6,8)	Lactic acid producing-bacteria	number of viable cells
Ganske and Bornscheuer[122]	$[C_4mIm][BF_4]$, $[C_4mIm][PF_6]$	*E. coli*, *Pichia pastoris*, and *Bacillus cereus*	EC_{50}
Pernak *et al.*[123]	C_nIm, $(OC_{n'})Im$ +lactates (n = 1-12, n'=4-12)	Gram-negative rods, Gram-positive cocci and fungi	MIC and MBC
Cornmell *et al.*[35]	$[P_{666(14)}][Tf_2N]$, $[N_{1888}][Tf_2N]$, $[P_{666(14)}][Cl]$, $[N_{1888}][Cl]$	*E. coli*	accumulation

Authors	Tested ionic liquids type(s)	Target(s)	Indicator/Method
Lee *et al.*[124]	$[C_nmIm]^+$ + $[PF_6]^-$, $[BF_4]^-$, $[C_1SO_4]^-$, $[SbF_6]^-$, $[Tf_2N]$, $^-$ $[CF_3SO_3]^-$ (n = 2,4,6,8) $[(C_3C_6H_6)mIm]^+$ + $[SbF_6]^-$, $[CF_3SO_3]^-$	*E. coli*	EC_{50}, growth rate
Pernak and Chwała[125]	$[N_{11R(OC6-18)}][Cl]$, R = $C_{1\ or\ 2}OH$, C_9COOC_1, $PhCOOC_1$	Cocci, rods and fungi	MIC
Myles *et al.*[126]	$[C_1COOC_nmIm]^+$ + $[Br]^-$, $[BF_4]^-$ (n =4,6,8)	8 bacteria and 12 fungi	MIC
Wang *et al.*[127]	$[C_4mIm]^+$, $[C_2OCmIm]^+$ + $[PF_6]^-$, $[BF_4]^-$, $[C_1SO_4]^-$, $[Tf_2N]^-$, $[CF_3SO_3]^-$	*Clostridium* sp.	Growth rate
Demberelnyamba *et al.*[128]	$[C_nmIm]^+$ + $[Cl]^-$, $[Br]^-$; $[(1\text{-}C_n)(2\text{-}m)(3\text{-}C_2OH)Im][Cl]$; $[C_n(C_2OH)Pyr]$	7 microbial strains	MIC
Cieniecka -Rosłonkiewicz[129]	$[P_{666n}]^+$ + $[Cl]^-$, $[Br]^-$, $[PF_6]^-$, $[BF_4]^-$, $[C_{n'}XO_2]^-$, $[Tf_2N]^-$, $[OTf]^-$, $[N(CN)_2]^-$, $[NO_3]^-$	13 bacteria or fungi	MIC and MBC
Petkovic *et al.*[130]	Im, Py, $[N_{111(2OH)}]$	filamentous fungi (*Penicillium* sp.)	metabolic footprint
Petkovic *et al.*[131]	$[N_{111(2OH)}][C_nCOO]$	4 fungal isolates	MIC
Babalola *et al.*[135]	$[Py][CrO_3Cl]$, $[Qu][CrO_3Cl]$	4 bacteria	MIC
Dipeolu *et al.*[136]	Im, N (hydroxyl)	*Clostridium sporogenes*	growth rate

3.1.2 Toxicity towards aquatic biological ecosystems: *Daphnia magna* and algae

The release of ionic liquids into the environment through accidental spills or as effluents can mainly affect the aquatic compartment due to their relatively high solubility. Consequently, aquatic toxicology investigations were the earliest topic of interest concerning potential ionic liquid hazard and many studies are now published. Moreover many biological recommended assays deal with aquatic systems to evaluate acute toxicity of chemicals.

A. Toxicity towards *Daphnia magna*

The freshwater crustacean *Daphnia magna* is the most popular live food for aquarium fish[106] and is therefore an important link between microbial and higher trophic levels. It is easily cultured in the laboratory and sensitive to xenobiotics. It is widely used to test the acute (effect on survival) and chronic toxicity (effect on reproduction) and even recommended as a international standard toxicity bioassay for animals by regulatory agencies (*e.g*., United States Environmental Protection Agency, OECD chronic assays).[115] The toxicity values reported are either LC_{50} (EC_{50}) corresponding to the concentration at which 50% of the exposed population are killed (Lethal concentration) or IC_{50} (EC_{50}) corresponding to the concentration at which 50% of the exposed population are immobilized.

Bernot *et al.*[137] have concluded that acute toxicity of imidazolium ionic liquids on *D. magna* (LC_{50}) occurred at concentrations comparable to those of organic solvents such as phenol, tetrachloromethane and trichloromethane, but at lower concentrations than benzene, methanol and acetonitrile. This classification depends on the alkyl side chain length and a strong correlation was found between this parameter and toxicity.[138,139] This correlation appeared also to be hold well for the pyridinium, phosphonium and ammonium-based ionic liquids.[138] In all these series, C_4 side chain compounds are moderately toxic whereas > C_6 ionic liquids are highly toxic. For the longer chain, the ionic liquids are many orders of magnitude more toxic than traditional organic solvents (Table 3.4).[138] These chemicals are comparable to surfactants and even their recoveries for analytical purposes are very poor.

Table 3.4. Acute toxicity of some organic solvents and ionic liquids on *D. magna*.[138]

Compounds	EC_{50} ($mg.L^{-1}$)	Compounds	EC_{50} ($mg.L^{-1}$)
Methanol	> 10 000	Dichloromethane	135
Toluene	11.5	Acetonitrile	> 100
$[C_4mIm][Cl]$	6.5	$[C_4Py][Cl]$	20
$[C_{12}mIm][Cl]$	0.0043	$[P_{66(13)(13)}][Cl]$	0.072
$[C_{18}mIm][Cl]$	0.0017	$[N_{88(17)(17)}][Tf_2N]$	0.2

The structure of the cations is the main factor influencing the toxicity, and the longer substituted alkyl chain on the cation increases again the toxicity of ionic liquids containing imidazolium, pyridinium or quaternary ammonium.[115,138] The number of nitrogen atoms in the aromatic cation ring has been shown to increase slightly the toxicity: ammonium < pyridinium < imidazolium < triazolium < tetrazolium. Conversely, toxicity is expected to decrease with ring methylation.[115] The introduction of one oxygen atom in the lateral chain of imidazolium ionic liquids results in a more than tenfold reduction in toxicity, a further increase in the number of oxygen atoms having no significant effect on toxicity.[117]

In almost all these cases, the counter-anion exhibits no or weak influence on toxicity,[12,115,132,137,138,139,140] except with oxygenated imidazolium ionic liquids where *D. magna* was found to be sensitive to the anion, $[BF_4]^-$ (significantly more toxic than $[N(CN)_2]^-$).[117]

Other toxicity studies have been carried out on samples obtained after biodegradation experiments of $[C_nmIm]^+$ (n = 4, 6, 8) by Docherty *et al.*[141] or on the new family of choline-based ionic liquids with saccharinate or acesulfamate as anions.[140] All these chemicals were found to have very low toxicity.

Luo *et al.*[142] have also monitored the development of *Daphnia magna* in the presence of $[C_8mIm][Br]$. The results show that the ionic liquid inhibited the growth of *D. magna* during the period of chronic exposure, but it could be restored when the crustaceans were no longer exposed to the toxicant. The same phenomenon was observed for reproductive parameters.

Table 3.5. Synthesis of published studies on ionic liquids toxicity towards *D. magna*.

Authors	Tested ionic liquids type(s)	Indicator/Method
Garcia *et al.*[12]	$[C_nmIm]^+ + [Cl]^-, [PF_6]^-, [BF_4]^-$ (n = 4,6,8)	IC_{50}
Couling *et al.*[115]	N, Py, Im, + triazolium, tetrazolium	EC_{50}
Samori *et al.*[117]	$[C_4mIm]^+, [(C_2O)_nC_1mIm]^+ + [BF_4]^-,], [N(CN)_2]^-$ (n =1- 4)	LC_{50}
Samori *et al.*[132]	$[C_4mIm][BF_4]$	EC_{50}
Ventura *et al.*[133]	$[C_3mIm][Tf_2N]$	EC_{50}
Bernot *et al.*[137]	$[C_4mIm]^+ + [Cl]^-, [Br]^-, [PF_6]^-, [BF_4]^-$	LC_{50}, Movement, feeding behavior
Wells and Coombe[138]	Im, Py, P, N + $[Cl]^-, [PF_6]^-, [C_1SO_4]^-, [Tf_2N]^-, [(C_2O)_2PO_2]^-$	EC_{50}
Yu *et al.*[139]	$[C_nmIm][Br]$ (n = 4,6,8,10,12)	LC_{50}
Nockemann *et al.*[140]	$N_{111(2OH)}$ +saccharinate and acesulfamate	EC_{50}
Docherty *et al.*[141]	Metabolites from $[C_nmIm][Br]$ (n = 4,6,8)	EC_{50}
Luo *et al.*[142]	$[C_8mIm][Br]$	LC_{50} , reproductivity

B. Toxicity towards algae

Algae are very important organisms for toxicology assessment because they are key part of aquatic ecosystems, playing the role of primary producers and providing energy to sustain higher trophic levels. Algal tests are generally sensitive, rapid and cost-effective and the corresponding bioassays are widely used as biological tools for environmental impact studies and are recommended by many organizations. They are generally based on the algal growth or photosynthesis inhibition. However, they are also a large and diverse group of eukaryotic aquatic photosynthetic organisms organized in different phyla. Numerous studies are reported on ionic liquid toxicity on different algae coming from marine or freshwater and the sensitivity of these organisms can be very different.[143] The comparison of the results obtained is therefore very difficult and has been attributed to different algal cell wall characteristics, known to play a critical role in the transport of materials, to morphological and physiological differences, to the use of dissimilar methods to evaluate the algal growth (optical density, fluorometry, etc), the exposure time to the ionic liquids (from 24h to several days), the nutrient medium used or even "environmental" parameters such as salinity, pH or temperature.[108]

Table 3.6 Examples of the $\log_{10}EC_{50}$ values (EC_{50} in µmol.L^{-1}) obtained with the acute toxicity towards algae for some ionic liquids (according to Petkovic *et al.*[108]).

Algae	*Selenastrum capricornutum*			*Scenedesmus vacuolatus*	*Scenedesmus quadricauda*	*Chlamydomonas reinhardtii*
Incubation time/h	48	72	96	24	96	96
$[C_4mIm][Br]$	3.46	3.36	3.02	2.25	1.34	3.69
$[C_6mIm][Br]$	2.57	2.54	2.46	0.08	-0.5	3.02
$[C_8mIm][Br]$	1.65	1.63	1.57	-2.67	-1.74	1.17

For example, Latała *et al.*[144,145] who selected two Baltic algae *Oocystis submarina* (green algae) and *Cyclotella meneghiniana* (diatom), found that the two species had very distinctive responses to $[C_4mIm]^+$, the growth of the first one being completely inhibited by the ionic liquid whatever its concentration and the second one having the ability to acclimatize and recover after 5 days. Moreover, it was discovered that the toxicity declined with the increase of salinity. This was absolutely not the case with two other diatoms: *Skeletonema marinoi* and *Phaeodactylum tricornutum*.[146] In this study, a big difference of sensitivity was also observed between the two species towards $[C_4mIm]^+$, $[C_2OCmIm]^+$ and $[C_2OC_2OCmIm]^+$. The toxicity could be associated with the silica uptake required for growing by *S. marinoi*, the most sensitive algae. The ionic liquids would play a role of scavenger of $[SiO(OH)_3]$ or interact with functional groups present on the cell surface, avoiding the formation of the siliceous cell wall.

The structure of the cation has a great influence on the toxicity. Conversely to the other assays presented before, the pyridinium ring presents a greater toxicity than the imidazolium one towards *Pseudokirchneriella subcapita*.[147] It decreases on going from aromatic heterocyclic nitrogen-containing compounds to non-aromatic cyclic and then acyclic compounds (pyrrolidinium > morpholinium > ammonium). Sulfonium and thiophenium-bases ionic liquids are practically harmless.[148]

Whatever the study, the trend of increasing toxicity with increasing alkyl chain length was also observed[138,149,150] as well as the decrease in toxicity by introducing an ether function.[146] The role of the counter-anion was more controversial: Matzke *et al.*[119] concluded that the anion effect was not as drastic as the side chain effect, whereas Cho *et*

al. [151] and Pham *et al.*[147] have described strong effect of $[PF_6]^-$ or $[BF_4]^-$ on *Pseudokirchneriella subcapita* due to their hydrolysis in water leading to fluoride formation. This algae has also shown greater sensitivity to $[Tf_2N]^-$.[148]

Table 3.7. Synthesis of published studies on ionic liquids toxicity towards algae.

Authors	Tested ionic liquids type(s)	Target(s)	Indicator/Method
Matzke *et al.*[119]	$[C_nmIm]^+$ + $[(CF_3)_2N_2]^-$, $[BF_4]^-$, $[Cl]^-$, $[C_8SO_4]^-$, $[Tf_2N]^-$, bis(1,2-benzenediolato)borate (n =2,4,8)	algae (*Scenedesmus vacuolatus*)	EC_{50}
Stolte *et al.*[120]	Py, Im, Mor, Pyr, N, (ether, hydroxyl and nitrile) + $[Tf_2N]^-$, $[Cl]^-$, $[Br]^-$, $[I]^-$	algae (*Scenedesmus vacuolatus*)	EC_{50}
Ventura *et al.*[133]	$[C_3mIm][Tf_2N]$	freshwater algae (*Pseudokirchneriella subcapitata* and *Chlorella vulgaris*)	EC_{50}
Wells and Coombe[138]	Im, Py, P, N + $[Cl]^-$, $[PF_6]^-$, $[C_1SO_4]^-$, $[Tf_2N]^-$, $[(C_2O)_2PO_2]^-$	freshwater algae	EC_{50}
Kulacki and Lamberti[143]	$[C_nmIm][Br]$ (n =4,6,8)	freshwater algae	EC_{50}
Latała *et al.*[144]	$[C_nmIm][BF_4]$ (n = 2,4)	baltic algae	inhibition growth
Latała *et al.*[145]	$[C_nmIm][Cl]$ (n = 2,4,6,8,10)	two green algae	inhibition growth
Samori *et al.*[146]	$[C_4mIm]$, $[C_2OCmIm]$, $[C_2OC_2OCmIm]$+ $[Cl]^-$	diatoms	EC_{50}
Pham *et al.*[147]	$[C_4mIm][Br]$, $[C_4mIm][BF_4]$	freshwater algae	EC_{50}
Pretti *et al.*[148]	Im, Py, N + $[Tf_2N]^-$	algae (*Pseudokirchneriella subcapitata*)	EC_{50}
Pham *et al.*[149]	$[C_nmIm][Br]$, $[C_nmPy][Br]$ (n = 3,4,6,8)	algae	EC_{50}
Cho *et al.*[150]	$[C_nmIm][Br]$ (n = 3,4,6,8)	freshwater algae	EC_{50}
Cho *et al.*[151]	$[C_4mIm]^+$+ $[SbF_6]^-$, $[PF_6]^-$, $[BF_4]^-$, $[CF_3SO_3]^-$, $[C_8SO_4]^-$, $[Br]^-$, $[Cl]^-$	microalgae	EC_{50}
Cho *et al.*[152]	Im, Py, Pyr, P, N + $[Br]^-$	microalgae	EC_{50}
Grabińska-Sota and Kalka[153]	$[C_nPy][Cl]$ (n =1-4)	algae	EC_{50}
Matzke *et al.*[154]	$[C_4mIm][BF_4]$, $[C_8mIm][BF_4]$, $[C_4mIm][Tf_2N]$, $CdCl_2$, mixtures of these compounds	algae (*Scenedesmus vacuolatus*)	EC_{20}, EC_{50}, EC_{80}
Ma *et al.*[155]	$[C_nmIm][Br]$ (n = 4,6,8,10,12)	green algae	EC_{50}

3.1.3 Enzyme inhibition and cytotoxicity

Ionic liquids can cause toxic effects towards cells by two types of mechanism: molecular toxicity and phase toxicity. Molecular toxicity represents the effects caused by ionic liquids dissolved in the aqueous phase and includes enzyme inhibition, protein denaturation and membrane modification such as damage or permeability changes. Phase toxicity effects include the extraction of nutrients, disruption of the cell wall (extraction of outer cellular components), and the limited access to nutrients caused by cell attraction to interfaces, the formation of emulsions and the coating of cells.[156] Phase toxicity can be caused by direct cell-solvent contact.

A. Enzyme inhibition

The potentialities of using enzymes as biomarkers of xenobiotics are well known and have been used more recently in studies with ionic liquid involving enzymes with biological impact. These assays evaluating enzymatic inhibition can present several advantages towards studies with a whole microorganism such as simplicity, duration of the assays and simplification of the data interpretation.[157] The difficulty is the choice of a pertinent enzyme that can bring valuable and representative data to assess the real xenobiotic impact.

Different enzymes have been tested such as catalyses involved in the oxidant stress metabolism[157], cytochromes P450, key enzymes involved in biodegradative pathways[158,159] but AMP-deaminase[160] and acetylcholinesterase have been the enzymes mainly used. We will focus on the results obtained with this last enzyme.

Acetylcholinesterase (AChE) is a critical enzyme of the nervous system that plays an important role in nerve signal conduction. It can be found in nearly all higher organisms with a highly conserved and well known active site.[161,162] This colorimetric assay is therefore the most effective and the quickest way to determine when chemicals are disturbing the normal body function. It was successfully used for organophosphate and carbamate pesticides and was first used to test ionic liquids due to the similarity of the chemical structure of most of the ionic liquids and of acetylcholine. The first detailed research works were carried out by the Jastorff group.[161,162] These authors monitored the

inhibition activity of purified AChE from electric eel (IC_{50} = concentration inhibiting 50% of AChE activity) in the presence of a wide range of ionic liquid cations and anions. They showed that: (i) the anion species has not a great influence on the activity of AChE. The fluoroanions exhibited a slightly higher toxicity than the other ones; (ii) the head groups can be classified according to their inhibition effect as pyridinium > imidazolium > piperidinium > pyrrolidinium > ammonium > phosphonium > morpholinium. This order can be due to stronger or weaker binding in the the anionic site; (iii) the increasing length of the alkyl side chain lead to a stronger inhibition. The lipophilicity of the chain, as well as its steric position, influences toxicity. That could be also explained by favourable or no interactions in the active site, located at the bottom of a narrow gorge. The introduction of polar functionalized side chains (ether or hydroxyl groups) exhibits a lower inhibitory potential than their lipophilic analogues: the presence, in particular of hydroxyl group, should repulsively prevent interaction between the quaternary nitrogen of ionic liquid and the anionic active site. The pyridinium-based ionic liquids containing a hydroxyl group and an ethereal side chain on the cation and saccharinate or acesulfamate as anions presented by Stasiewicz *et al.*[163] did not affect AChE activity.

Recently, some authors have monitored AChE activity directly in living organism. Luo *et al.*[164] have shown that AChE of earthworm was very sensitive, in particular of the long alkyl chain of imidazolium-based ionic liquid ($[C_8mIm]^+$). A decrease of AChE activity was monitored during the first 3 days of exposure and then the activity increased to be very similar than that of the blank after 7 days of exposure. No explanation can be found. This result suggests that ionic liquid could be absorbed through the worm skin or gut. The use of AChE from rat cell line CP12[117] confirms that ionic liquids interact with biological membranes as an inhibition of this enzyme activity was also evidenced in the case of long alkyl side chain. The same experiment carried out with oxygenated cation presents no inhibition, independently to the anion and the number of oxygen present in the lateral chain.

B. Cytotoxicity / Cell viability

Many studies investigated the effects of various ionic liquids in different human and

animal cell lines, such as human breast cancer cells MCF7[165], human cells[166,167,168,169,170] and rat cells.[113,171] The more frequently used cells in cytotoxicity tests with ionic liquids are rat promyelotic leukaemia cell line IPC-81 and human cell lines such as HeLa (human tumor cells). In general, the cells are incubated in an adapted medium together with the ionic liquid at different concentrations, in a 96-well plate. After a specified duration at a stable temperature, cell viability was calculated as the percentage of the viability of the exposed cells *vs* control by measure of absorbance.

All these studies observed a strong correlation between toxicity and alkyl side chain length for imidazolium, pyridinium and quaternary ammonium ionic liquid family, with low toxicity for carbon chain < C_6. The increase in the lipophilicity was suggested to be linked to a better cellular sorption (adsorption to the membrane and uptake into the cell) and a stronger cytotoxicity.[113,172] Nevertheless the low effect of short chain depends on their concentration and at high concentration, a high cytotoxicity has been found. The mode of toxicity seems to involve disruption of the cell membrane, correlated to cellular apoptosis and even necrosis.[166] It was observed that the incorporation of a polar side chain (ether, hydroxyl and nitrile) decreases the cytotoxicity.[117,163,165,172,173] Several studies revealed also that both the cation and the anion structures contribute to cell cytotoxicity with strong effect of the fluorous anions due to partial hydrolysis of $[BF_4]^-$ and $[PF_6]^-$ into fluoride anions.[171] Conversely, Ranke *et al.*[113] have pointed out that $[BF_4]^-$, $[PF_6]^-$, $[Cl]^-$ anions do not show any systematic effect on the toxicity, whereas the effect of anions remains unclear in the work of Stepnowski *et al.*[167] From the Quantitative Structure-Activity Relationship (QSAR) study of Garcia-Lorenzo *et al.*[170], the anion methyl or ethyl sulfate presents a negative contribution to the toxicity, while the $[Cl]^-$ gives a positive contribution. The choice of benign anions (saccharinate or acesulfamate) combined with a pyridinium core substituted with polar chains may constitute an advance in the design of green ionic liquids as these compounds present no cytotoxicity to the rat cell line IPC-81.[163]

Compared to the traditional organic compounds, the EC_{50} values determined for the ionic liquids analysed in the studies in cells are lower (Table 3.8).

Table 3.8. Cytotoxicities data of some common compounds and ionic liquids (EC_{50}/mmol.L^{-1}).

Chemicals	IPC-81	HeLa	Chemicals	IPC-81	HeLa
$NaBF_4$	> 4[171]	14.33[166]	Xylene[174]		355
$Na(CN)_2N$	14[171]		Methanol[174]	1600	646
$NaC_8H_{17}SO_4$	3[171]		Phenol[174]		5.2
NaF	1.2[171]		Ethanol[174]	700	302
NaBr	>5[171]		Propanol	100[173]	
NaI	>5[171]		$LiTf_2N$		4.24[166]
$[C_4mIm][BF_4]$	1.7[171]	4.55[166]	$[C_6mIm][Tf_2N]$	0.18[171]	
$[C_4mIm][Tf_2N]$	0.48[171]	1.17[166]	$[C_4mPy][Tf_2N]$		1.68[166]

The differences observed between the results obtained in the different studies can be due to the different cell lines used but mostly on the exposure time that can go from 4h to 3-5 days.

Table 3.9. Synthesis of published studies on Acetylcholine esterase inhibition and cytotoxicity of ionic liquids.

Authors	Tested ionic liquids type(s)	Target(s)
Ranke *et al.*[53]	Im, Pyr, Py, P, N and Qu + $[Cl]^-$, $[Br]^-$, $[BF_4]^-$, $[PF_6]^-$	IPC-81
Ranke *et al.*[113]	$[C_nmIm]^+$+ $[PF_6]^-$, $[BF_4]^-$, $[Cl]^-$, $[Br]^-$ (n = 3-10)	WST-1
Samori *et al.*[117]	$[C_4mIm]^+$, $[(C_2O)_nC_1mIm]^+$+ $[BF_4]^-$, $[N(CN)_2]^-$ (n = 1-4)	AChE, rat cell line PC12
Matzke *et al.*[119]	$[C_nmIm]^+$+ $[(CF_3)_2N_2]$, $[BF_4]^-$, $[Cl]^-$, $[C_8SO_4]^-$, $[Tf_2N]^-$, bis(1,2-benzenediolato)borate (n = 2,4,8)	AChE
Składanowski *et al.*[160]	N-glucopyranosyl ammonium salts, $[C_4mIm]^+$ + $[PF_6]^-$, $[BF_4]^-$, $[Cl]^-$, *p*-tosylate	AMP deaminase
Stock *et al.*[161]	Im, Py, P + different anions	AChE
Arning *et al.*[162]	Py, Im, Mor, Pyr, Pip, (ether, hydroxyl) + halides anions	AChE
Stasiewicz *et al.*[163]	$(C_1OC_n)(OH)Py$ + $[Cl]^-$, acesulfamates, saccharinates (n = 3-7, 11)	AChE and IPC-81
Luo *et al.*[164]	$[C_nmIm][Br]$ (n = 4,6,8,10,12)	AChE (earthworm)
Kumar *et al.*[165]	Py, Pyr, Pip, Im + $[Br]^-$, $[Tf_2N]^-$, $[BF_4]^-$, $[TfO]^-$, $[AsF_6]^-$, $[N(CN)_2]^-$	MCF7
Wang *et al.*[166]	Im, Py, $P_{(14)RRR}$, N_{222R}, $N_{11(2OH)R}$ + $[BF_4]^-$, $[Cl]^-$, $[Br]^-$, $[Tf_2N]^-$ R = $C_{2, 4, 8}$, $C_1C_6H_5$, $C_2{=}C_1$	HeLa
Stepnowski *et al.*[167]	Im + different anions	HeLa
Frade *et al.*[168]	Im (ether, hydroxyl) + $[Cl]^-$	HT-29 and CaCo-2
Frade *et al.*[169]	Im, Py, Pyr, N, P, guanidinium, + dicyanoamide, $[Tf_2N]^-$	Caco-2
Garc á-Lorenzo *et al.*[170]	$[C_nmIm]^+$, $[(aryl)mIm]^+$ + $[PF_6]^-$, $[Cl]^-$, $[C_{n'}SO_4]^-$ (n = 4,6,8,10, n' = 1,2)	Caco-2
Stolte *et al.*[171]	$[C_nmIm]^+$ + 27 anions (n = 2,4,6)	IPC-81, WST-1
Stolte *et al.*[173]	Py, Im, Mor, Pyr, N, (ether, hydroxyl and nitrile) + $[Tf_2N]^-$, $[Cl]^-$, $[Br]^-$, $[I]^-$	IPC-81, WST-1
Jodynis-Liebert *et al.*[175]	$[]N_{11(10)(10)}$][saccharinate]	A549, HepG2, LoVo, DLD-1,AGS, HaCaT
Zhang *et al.*[176]	Guanidinium + $[PF_6]^-$, $[BF_4]^-$, $[Cl]^-$, $[Br]^-$	HeLa, B16 and SMMC-7721
Salminen *et al.*[177]	Pyr, Pip + $[Br]^-$	MCF7

3.1.4 Toxicity towards terrestrial plants

The published studies on the phytotoxicity of ionic liquids mainly focus on the aquatic ecosystems (see Section 3.1.2), but very little data is available on the ecotoxicological effects of ionic liquids on terrestrial plants. The imidazolium-based ionic liquids,[178,179] including the chiral ones,[180] have potential toxic effect on the growth development and germination of plants, dependant chiefly on the applied concentration.

Low concentrations (*ie.* 0.4 mmol.L^{-1} [C_4mIm][BF_4][178], 0.01 - 0.1 mg.L^{-1} [$C_{2\text{-}6}$mIm][Cl] [179], 100 mg.kg^{-1} dry soil [(-)-1-[(1*R*)-Nopyl]-mIm][Cl][180]) were safe for the plants, in some case even promoted the growth of plants.[178]

Wang *et al.*[178] have studied the phytotoxicity of [C_4mIm][BF_4] - which has been reported having low toxicity towards algae – to the wheat *Triticum aestivum*. They figured out that wheat germination was reduced to 38.0% in the presence of 4.4 mmol.L^{-1} [C_4mIm][BF_4] compared to 100% germication in the control. Similarly, the root and shoot length of wheat seedlings and the chlorophyll content decreased with increasing concentrations of the ionic liquid. The activities of amylase, peroxidase and the soluble protein content increased in shoots and roots, but decreased significantly in germinating seeds.

The influences of the cation and anion species are not continuous, depending on the soil type. In most of the cases, the trend of increasing toxicity towards wheat and garden cress with increasing alkyl chain length was observed,[179,181] but [C_2mIm][BF_4] was more toxic than [C_4mIm][BF_4] for the wheat.[181] Matzke *et al.*[182] have studied the influence of four different anion species of [C_nmIm] based ionic liquids on the toxicity towards wheat and cress. The toxicity of [C_4mIm][Cl], [C_4mIm][BF_4] and [C_4mIm][HSO_4] was mainly dominated by the cationic moiety, varying in dependence of the added clay type and clay concentration. However [C_4mIm][Tf_2N] appeared the most toxic independently of the investigated soil type.

The morphology of diverse plant classes is different, for example, the roots of monocotyledonous plants are adventitious and they do not perform secondary growth.[181] Thus, differences in sensitivity between the monocotyledonous and dicotyledonous plants could be observed. In the study of Bałczewski *et al.*[180], the lowest concentration of the

chiral ionic liquids causing a distinct reduction in plant germination growth to common radish (dicotyledonous) was 100 $mg.kg^{-1}$, but was 200 $mg.kg^{-1}$ to spring barley (monocotyledonous).[180] However, the differences of sensitivity were not consistent and variable within wheat and watercress.[181] So at present it is not possible to generate general statements on future testing strategies for monocotyledonous and dicotyledonous plants regarding their species sensitivity.

The soil properties may also influence the bioavailability and phytotoxicity of xenobiotics. Matzke *et al*. have investigated the role of organic matter content of two types of clay minerals (smectite and kaolinite) - on the phytotoxicity of the imidazolium-based ionic liquids with different anion species[182] and alkyl side chain length.[181] They observed a decrease of toxicity with increasing the organic matter content. The same effect was observed by Studzinska and Buszewski[179] in their study towards garden cress in five soils with different TOC (total organic carbon) content. In other words "the more fertile the soil, the lower probability of hazardous effects of ionic liquids to plants". This is probably because the soil containing more organic matter sorbs the ionic liquids in a more extensive way, hence less ionic liquid is absorbed by plants. The influence of the clay minerals varied according to its type and concentration. Overall the influence of the 2:1 layer mineral smectite on toxicity was stronger than the 1:1 layer mineral kaolinite. An increase of clay content resulted in less pronounced toxic effects of these substances. In contrast, for $[C_4mIm][Tf_2N]$, the addition of clay minerals caused higher toxic effects in comparison to the reference soil.

Table 3.10. Synthesis of published studies on ionic liquids toxicity to plants.

Authors	Tested ionic liquids type(s)	Target(s)	Indicator/Method
Matzke *et al.*[119]	$[C_nmIm]^+$ + $[(CF_3)_2N_2]^-$, $[BF_4]^-$, $[Cl]^-$, $[C_8SO_4]^-$, $[Tf_2N]^-$, bis(1,2-benzenediolato)borate (n = 2,4,8)	wheat (*Triticum aestivum*), cress (*Lepidium sativum*), duckweed (*Lemna minor*)	growth inhibition
Stolte *et al.*[120]	Py, Im, Mor, Pyr, N, (ether, hydroxyl and nitrile) + $[Tf_2N]^-$, $[Cl]^-$, $[Br]^-$, $[I]^-$	*Lemna minor*	EC_{50}
Wang *et al.*[178]	$[C_4mIm][BF_4]$	wheat	growth inhibition and impair
Studzińska and Buszewski[179]	$[C_nmIm][Cl]$ (n = 2,4,6)	watercress	relative growth, absorbed by plan, effect of soil organic matter
Bałczewski *et al.*[180]	[(-)-1-[(1*R*)-Nopyl]-mIm]$^+$ + $[Cl]^-$, $[NO_3]^-$; mIm	spring barley radish	growth and germination inhibition
Matzke *et al.*[181]	$[C_nmIm][BF_4]$ (n = 2,4,8)	wheat and watercress	growth inhibition effect of soil composition
Matzke *et al.*[182]	$[C_4mIm]^+$ + $[Cl]^-$, $[BF_4]^-$, $[Tf_2N]^-$, $[HSO_4]^-$	wheat	growth inhibition effect of soil composition

3.1.5 Toxicity towards animals

A. Effects on invertebrates

The published ecotoxicological studies on the invertebrates *Daphnia magna* has been overviewed in the previous part "Toxicity towards aquatic biological ecosystems". The other studies mainly focus on the survival, growth and reproductive ability of the invertebrates in either soil or water.

Swatloski and coworkers[183] have observed that lengthening the alkyl chain on cation of [C_nmIm][Cl] ionic liquids led to a concomitant increase in toxicity/lethality to *Caenorhabditis elegans*, a soil nematode, presumably through surfactant/detergent effects: when the worms were exposed to 1.0 mg/mL ionic liquid the lethality went from 0.0% with [C_4mIm][Cl], to 11% with [C_8mIm][Cl], to 97% with [C_{14}mIm][Cl]. A similar tendence has been observed in the studies on [C_nmIm][Br] towards the earthworm *Eisenia foetida*[164], as well as on the [C_nmIm] and [C_nmPy] based ionic liquids towards a freshwater pulmonate snail *Physa acuta* (LC_{50} = 1 - 325 $mg.L^{-1}$)[184] and on the zebra mussel *Dreissena ploymorpha* (LC_{50} = 21.4 - 1290 $mg.L^{-1}$)).[185]

In another study of the group of Luo *et al.*[186], the toxic effect of [C_8mIm][Br] on the growth and reproductive ability - which are more sensitive indicator than survival - has been studied. [C_8mIm][Br] inhibited the growth reproductive ability of *E. foetida*, depending on the ionic liquid concentration and exposure time.

Bernot *et al.*[184] have observed that snails moved more slowly when exposed to C_4 and C_6- imidazolium and pyridinium cation ionic liquids at 1 to 3% of LC_{50} but movement was not affected at 4–10% of LC_{50}, neither by C_8 ionic liquids. The snails grazing patterns and egestion rates were also affected. The feeding rate of molluscs was also reduced in short-term exposure of any ionic liquids tested in the study of Costello *et al.*[185]

Costello *et al.*[185] have compared the toxicity towards zebra mussel of their tested ionic liquids to traditional solvents, and they concluded that the ionic liquids with butyl alkyl chains had similar toxicities to phenol, whereas octyl alkyl chains ionic liquids were as toxic as hydrazine.

B. Effects on vertebrates

The currently published studies on the toxicity of ionic liquids towards vertebrates are far from enough. They focus on fishes and rodents.

Fishes are important consumers in the aquatic food chain, and they play a key role in keeping aquatic ecosystem balanced. Pretti *et al.*[187] have evaluated the lethality of 15 widely used ionic liquids with different cations to Zebrafish *Danio rerio*, a prominent model vertebrate. The tested immidazolium, pyridinium and pyrrolidinium ionic liquids had 96h LC_{50} values greater than 100 $mg.L^{-1}$, whereas the ammonium salts $[N_{1(cocos)[C2(OC2)nOH][C2(OC2)mOH]}][C_1SO_4]$ and $[N_{12(stearyl)(stearyl)}][Cl]$ had LC_{50} values of 5.2 and 5.9 $mg.L^{-1}$ respectively. These values were remarkably lower than the LC_{50} data reported for organic solvents and tertiary amines.

Wang *et al.*[188] have determined the toxicity of $[C_8mIm][Br]$ on goldfish (*Carasinus auratus*) early embryonic development. $[C_8mIm][Br]$ treatment also caused remarkable increases of embryonic malformation and mortality ratio in most treatment groups. The toxicity of $[C_8mIm][Br]$ on fish embryos showed a dose-response correlation and developmental stage-specific and the 72h-LC_{50} at different stages ranged 187 - 298 $mg.L^{-1}$. Similarly, the developmental toxicity to an amphibian, the frog *Rana nigromaculata*, was also stage sensitive and the 96h-LC_{50} at different stages ranged 42 - 85 $mg.L^{-1}$.[189]

Concerning the mammals, some authors have studied the effects of ionic liquids on rodents and determined acute and subacute toxicities after different types of administration (intravenous, oral, dermal). The lethal concentrations to rats reported in literature are listed in Table 3.11.

Table 3.11. LD_{50} of some ionic liquids to rats.

Chemicals	animals	$LD_{50}/mg.kg^{-1}$
dimethylformamide[190]	rat	2800
$[C_4mIm][Cl]$[191]	female Fischer 344 rats	550
$[C_1OC_6mIm][BF_4]$[192]	Wistar rats	female 1400; male 1370
$N_{11(10)(10)}$ saccharinate[175]	Wistar rats	>500
$N_{11(10)(10)}$ acesulfamate[193]	Wistar rats	>500

The group of Sipes and coworkers has characterized the effect of dose and route of administration on the disposition and elimination of [C_4mIm][Cl][194] and [C_4mPy][Cl][195] in rats and/or mice. This was administrated to rats at 175 mg.kg [C_4mIm][Cl] by oral gavage survived and appeared active and healthy. After intravenous 5mg.kg^{-1} or oral 50mg.kg^{-1} administration of ^{14}C labeled ionic liquids, the compounds are rapidly (within 48h) cleared from the systemic circulation and excreted unchanged in the urine. The total ^{14}C remaining in the liver, kidneys and lung aftert 72h was less than 0.01%. In all the urine and blood samples, only the parent compound was detected. These studies illustrate that systemic bioavailability of [C_4mIm][Cl] and [C_4mPy][Cl] is high, tissue disposition and metabolism are negligible, and absorbed compound is extensively extracted by the kidney and eliminated in the urine as the parent compound.[194,195]

The acute and subacute oral toxicity of [$N_{11(10)(10)}$][saccharinate][175] and acesulfamate[193] in rats have been investigated. No treatment-related microscopic changes were observed. In acute toxicity experiments, both ionic liquids introduced organ pathology, included exfoliation of the surface layer of the alveolar septa in lung parenchyma. In subacute experiments, reduced body weight gain, reduced food consumption, hematology changes were found. In addition, they also caused statistically significant changes in clinical chemistry parameters such as GGT and SDH activity, and decreases in glucose concentration. Under the conditions of this study, the lowest-observed-adverse-effect level of the two ionic liquids was considered to be 10 mg/kg/day.

The exposure of mice to ionic liquid [C_4mIm][Cl] and [C_{10}mIm][Cl] affected their reproductive parameters.[196] In addition, significantly decrease of fetal weight and a low incidence of apparent teratogenic effect, were associated with both [C_4mIm][Cl] and [C_{10}mIm][Cl]. No morphological defects were observed in any of the [C_2mIm][Cl] treated groups, despite maternal morbidity at the highest dosage level. These results strongly support the supposition that the toxicity of imidazolium-based ionic liquids is influenced by alkyl chain length.[197]

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Table 3.12. Synthesis of published studies on ionic liquids toxicity to animals.

authors	tested ionic liquids type(s)	target(s)	Indicatot/Method
Matzke *et al.*[119]	$[C_nmIm]^+$+ $[(CF_3)_2N_2]^-$, $[BF_4]^-$, $[Cl]^-$, $[C_8SO_4]^-$, $[Tf_2N]^-$, bis(1,2-benzenediolato)borate (n = 2,4,8)	spring tail (*Folsomia candida*)	reproduction inhibition
Ventura *et al.*[133]	$[C_3mIm][Tf_2N]$	cladocerans (*Daphnia longispina*)	EC_{50}
Luo *et al.*[164]	$[C_nmIm][Br]$ (n = 4,6,8,10,12)	earthworms	LC_{50} at different stages, AChE and cellulase activities
Swatloski *et al.*[183]	$[C_nmIm][Cl]$ (n =4,8,14)	soil roundworm (*Caenorhabditis elegans*)	lethality
Bernot *et al.*[184]	Im and py + $[Br]^-$	freshwater pulmonate snails (*Physa acuta*)	LC_{50}, Movement, feeding behavior
Costello *et al.*[185]	$[C_nmIm][Br]$, $[C_nmPy][Br]$ (n = 4,6,8)	zebra mussel (*Dreissena polymorpha*)	mortality and feeding, LC_{50}
Luo *et al.*[186]	$[C_8mIm][Br]$	earthworms	growth, reproductivity, and ATPase activity
Pretti *et al.*[187]	Im, Py, Pyr and N (hydroxyl) $]^+$ + $[BF_4]^-$, $[Cl]^-$, $[C_{1\ or\ 2}SO_4]^-$, $[PF_6]^-$, $[Tf_2N]^-$, $[N(CN_2)_2]^-$, $[H_2PO_4]^-$, $[NO_3]^-$	zebrafish *(Danio rerio*)	LCs
Wang *et al.*[188]	$[C_8mIm][Br]$	Goldfish (*Carassius auratus*)	LC_{50}, embryonic development
Li *et al.*[189]	$[C_8mIm][Br]$	frog	LC
Landry *et al.*[191]	$[C_4mIm][Cl]$	rats	LD_{50}
Jodynis-Liebert *et al.*[175]	$N_{11(10)(10)}$ saccharinate	rats, human cell lines	cytotoxicity, acute and subchronic oral toxicity
Jodynis-Liebert *et al.*[193]	$N_{11(10)(10)}$ acesulfamate	rats	LD_{50}, subchronic toxicity
Sipes *et al.*[194]	$[C_4mIm][Cl]$	rats	effect of dose and route of administration on the
Cheng *et al.*[195]	$[C_4py][Cl]$	rats	disposition of ionic liquid
Bailey *et al.*[196]	$[C_4mIm][Cl]$	CD-1 mice	influence on fetuses
Bailey *et al*[197]	$[C_nmIm][Cl]$ (n = 2,4,10)	CD-1 mice	fetal weight, teratogenic effect

3.1.6 Conclusion

There is a growing interest on the environmental impact and fate of ionic liquids, due in particular to more severe legislation and restriction. Ionic liquids are tunable chemicals by the various choices of the (cation + anion) combinations. Their potential to conceptually fulfil the requirements of environmental sustainability is remarkable – a balance has to be found between their interesting physico-chemical properties for diverse applications and their eco-friendly behavior.

The difficulty - and it is the case for all the chemicals - is the evaluation of the environmental impact and toxicity. The choice of the biological reference can be crucial. Up to now, a broad range of testing models – bacteria, fungi, algae, plants, crustacean, mammalian cell lines and animals – has been used.

The few studies, that have compared the response of different biological level models[119], have shown large difference in the EC_{50} values obtained with different organisms, suggesting species-dependant mechanisms of action. Similarly, the assays to evaluate the biodegradability of ionic liquids and to define them as “readily biodegradable” lead to disperse results. According to the test used, a compound can be put in the “readily biodegradable” classification or not.

The understanding of the detailed mechanisms is still lacking. Some information has been brought by detailed studies of the biodegradation pathways of some ionic liquids but they are still rare.

Nevertheless all these studies have shown the relatively high toxicity and low biodegradability of ionic liquids with long alkyl side chains cations due to the destabilization or even damage of the biological membrane linked to high lipophilicity . The introduction of oxygenated functionalization leads to a decrease of toxicity. The pyridinium ring is usually more biodegradable than the imidazolium one. The counter-anion has in most cases a minor or no effect.

Table 3.13. Toxicity (EC_{50}/µmol.L^{-1}) of non-functionalized ionic liquids toward different levels of biological complexity including enzyme (Acetyl Choline Esterase AChE), bacteria (*V. fischeri*, *E. coli*), algae (*Pseudokirchneriella subcapitata* = *P. s* and *Scenedesmus vacuolatus* = *S. v*), duckweed (*L. minor*), invertebrate (*D. magna* = *D. m*) and cell lines from rat (IPC 81) and from human (Hela, MCF7).

Ionic liquids	*V. fischeri*	*E. coli*	AChE	IPC-81	Hela	MCF7	*P. s*	*S. v*	*D .m*	*L. minor*
$[C_2mIm][Cl]$	35481[118]		115[198]					603[120]		
	21380[120]									
$[C_2mIm][BF_4]$		177828[124]	112[198]	2754[198]	10000[166]					
$[C_2mIm][PF_6]$			112[198]	8318[198]						
$[C_2mIm][Tf_2N]$			107[198]		1820[166]					
$[C_3mIm][Cl]$			186[198]	>19953[198]						
$[C_3mIm][BF_4]$	8710[113]		107[161]		2951[198]					
$[C_3mIm][PF_6]$			166[198]		>1000[198]					
$[C_4mIm][Cl]$	5129[115]		81[119]	3548[198]			219[138]	182[119]	85[115]	661[119]
	912[116]								85[137,]	
	2188[12]									
	2951[120]									
$[C_4mIm][Br]$	10233[115]		79[161]	2692[198]	2754[166]		2884[149]		37[115]	
	1175[113]								36[137]	
	2291[116]								71[139]	
	1862[12]									
$[C_4mIm][BF_4]$	3548[113]	39811[124]	95[119]	1318[198]	5248[166]			129[119]	48[115]	316[199]
	1259[12]				4571[166]				47[137]	
	1318[132]									

Ionic liquids	*V. fischeri*	*E. coli*	AChE	IPC-81	Hela	MCF7	*P. s*	*S. v*	*D .m*	*L. minor*
$[C_4mIm][PF_6]$	1175[12]	14125[124]	141[161]	1259[198]	13804[166]		158[138]		71[115,137]	
$[C_4mIm][C_8H_{17}SO_4]$				1700[171]						
$[C_4mIm][Tf_2N]$	2455[115]	355[124]	91[119]	479[198]	1175[166]		63[148]	65[119]		282[119]
$[C_4mIm][I]$				3000[171]						
$[C_4mIm][(CN)_2N]$	4677[115]		89[161]	1413[198]						
	1000[116]									
$[C_5mIm][Cl]$			91[198]	>1000[198]						
$[C_5mIm][BF_4]$	1380[113]		72[198]	>1000[198]						
$[C_5mIm][PF_6]$			71[198]	>1000[198]						
$[C_6mIm][Cl]$	87[118]		83[198]	708[198]			0.012[138]	1.2[120]		
	209[12]									
	813[113]									
$[C_6mIm][Br]$	26[115]						372[149]		6[115]	
	7[116]								11[139]	
$[C_6mIm][BF_4]$	1514[113]		76[198]	955[198]						
$[C_6mIm][PF_6]$	148[12]	1778[124]	76[198]	813[198]						
$[C_6mIm][Tf_2N]$		339[124]	141[198]	174[198]		661[165]				
$[C_7mIm][Cl]$			117[198]	427[198]						
$[C_7mIm][BF_4]$	275[113]		132[198]	380[198]						
$[C_7mIm][PF_6]$			81[198]	200[198]						
$[C_8mIm][Cl]$	15[12]		40[198]	102[198]			0.035[138]	0.002[120]		
	10[120]									

Ionic liquids	*V. fischeri*	*E. coli*	AChE	IPC-81	Hela	MCF7	*P. s*	*S. v*	*D .m*	*L. minor*
$[C_8mIm][Br]$	4[115]				302[166]		45[149]		0.047[115]	
	1.2[116]								3[139]	
$[C_8mIm][BF_4]$	26[113]		34[119]	39[198]	302[166]	708[165]		0.05[119]		8[199]
$[C_8mIm][PF_6]$	9[12]	437[124]	107[198]	91[198]						
$[C_8mIm][Tf_2N]$			107[198]	44[198]	191[166]					
$[C_9mIm][BF_4]$	5[113]									
$[C_{10}mIm][Cl]$	3[113]		12[198]	22[198]				0.0003[120]		
	0.6[120]									
$[C_{10}mIm][BF_4]$	0.7[113]		13[161]	6[198]						
$[C_{10}mIm][PF_6]$			48[198]	32[198]						
$[C_{14}mIm][Cl]$	0.7[120]		3[198]	0.4[198]				0.003[120]		
$[C_{16}mIm][Cl]$	2[120]		5[198]	0.6[198]				>0.01[120]		
$[C_{18}mIm][Cl]$	28[120]		9[198]	1[198]				>0.01[120]		
$[C_{19}mIm][Cl]$			23[198]	25 [198]						
$[C_{19}mIm][BF_4]$			27[198]	45[198]						
$[C_{19}mIm][PF_6]$			42[198]	71[198]						
$[C_2eIm][Br]$			120[198]	>1000[198]						
$[C_3eIm][Br]$			162[198]	>1995[198]						
$[C_4eIm][BF_4]$	631[113]		107[161]	1820[198]	22909[166]					
$[C_5eIm][BF_4]$	1380[113]									
$[C_6eIm][Br]$			59[198]	102[198]						
$[C_6eIm][BF_4]$	141[113]		69[198]	182[198]						

Ionic liquids	*V. fischeri*	*E. coli*	AChE	IPC-81	Hela	MCF7	*P. s*	*S. v*	*D .m*	*L. minor*
$[C_{10}eIm][Br]$			8[198]	3[198]						
[Py][Cl]			>1000[198]							
$[C_2Py][Cl]$			126[198]							
$[C_2Py][Br]$					9570[166]					
$[C_2Py][Tf_2N]$					2570[166]					
$[C_3Py][Br]$			166[198]							
$[C_3Py][Tf_2N]$			162[198]							
$[C_4Py][Cl]$	2570[115]		50[198]				372[138]	389[120]		209[120]
	447[116]									
	1514[120]									
$[C_4Py][Br]$	2512[115]		59[198]	7943[198]	3162[166]					
	550[116]									
$[C_4Py][BF_4]$			63[198]	1514[198]						
$[C_4Py][PF_6]$			69[198]							
$[C_4Py][(CN)_2N]$	2042[115]									
	417[116]									
$[C_4Py][Tf_2N]$					1670[166]					
$[C_5Py][Br]$			33[198]							
$[C_5Py][Tf_2N]$			35[198]							
$[C_6Py][Cl]$			52[198]							
$[C_6Py][Br]$									12[115]	
$[C_6Py][PF_6]$			58[198]							

Ionic liquids	*V. fischeri*	*E. coli*	AChE	IPC-81	Hela	MCF7	*P. s*	*S. v*	*D .m*	*L. minor*
$[C_6Py][Tf_2N]$			71[198]							
$[C_8Py][Cl]$			40[198]	19[198]						
$[C_8Py][Br]$					280[166]					
$[C_8Py][Tf_2N]$			25[198]							
$[C_4(2\text{-m})Py][Cl]$			5[198]							
$[C_4(2\text{-m})Py][BF_4]$			7[198]	1778[198]						
$[C_4mPy][Cl]$			14[198]							
$[C_4mPy][Br]$	562[115]						2884[149]		58[115]	
	135[116]									
$[C_4mPy][BF_4]$			34[161]	1995[198]						
$[C_4mPy][PF_6]$			28[161]							
$[C_4mPy][(CN)_2N]$	457[115]		17[198]							
	100[116]									
$[C_6mPy][Cl]$	28[118]		11[198]							
$[C_6mPy][Br]$	115[115]								4[115]	
	31[116]									
$[C_8mPy][Cl]$			4[198]							
$[C_8mPy][Br]$	6[115]								3[115]	
	2[116]									
$[C_6(4\text{-m})Py][Cl]$			28[198]							
$[C_6(4\text{-m})Py][BF_4]$			30[198]	148[198]						
$[C_8(4\text{-m})Py][Cl]$			13[198]	43[198]						

Ionic liquids	*V. fischeri*	*E. coli*	AChE	IPC-81	Hela	MCF7	*P. s*	*S. v*	*D .m*	*L. minor*
$[C_8(4\text{-m})Py][BF_4]$			17[198]	31[198]						
$[C_4mPyr][Cl]$	>19953[120]		83[198]	>19953[198]				2344[120]		145[120]
$[C_4mPyr][Br]$			85[198]	5888[198]		43652[177]	4677[149]			
						18197[165]				
$[C_4mPyr][BF_4]$			81[198]	794[198]						
$[C_4mPyr][Tf_2N]$			135[198]	1023[198]		1413[165]	>240[148]	339[120]		955[120]
$[C_4mPyr][(CN)_2N]$			95[198]	16982[198]						
$[C_6mPyr][Cl]$	977[198]		302[198]	813[198]						
$[C_6mPyr][Tf_2N]$			398[198]							
$[C_8mPyr][Cl]$			229[198]	389[198]						
$[C_8mPyr][BF_4]$			105[198]	66[198]						
C_6hPyr			120[198]	17[198]						
$[C_4mMor][Cl]$	>19953[120]									
$[C_4mMor][Br]$			513[198]	>19953[198]				>10000[120]		1288[120]
$[C_4mMor][Tf_2N]$			603[198]	2692[198]				100[120]		1413[120]
$[C_4mPip][Br]$	18621[120]		68[198]	10715[198]		14125[177]		1862[120]		3[120]
$[C_4mPip][Tf_2N]$			60[198]	2570[198]		871[165]		120[120]		708[120]
$[C_4Qu][Br]$			6[198]	209[198]						
$[C_4Qu][BF_4]$			4[198]	145[198]						
$[C_6Qu][BF_4]$			3[198]	12[198]						
$[C_8Qu][Br]$				0.9[198]						
$[C_8Qu][BF_4]$			2[198]	1.5[198]						
$[N_{1111}][Br]$	>100000[115]									

Ionic liquids	*V. fischeri*	*E. coli*	AChE	IPC-81	Hela	MCF7	*P. s*	*S. v*	*D .m*	*L. minor*
$[N_{1114}][Tf_2N]$			398[198]	4074[198]						
$[N_{1123}][Tf_2N]$			219[198]							
$[N_{1124}][Cl]$			115[198]	>19953[198]				>10000[120]		7[120]
$[N_{1124}][Tf_2N]$			107[198]	2692[113]				60[120]		
$[N_{2222}][Cl]$			631[198]	>3020[198]						
$[N_{2222}][Br]$	>100000[115]				18197[166]					
$[N_{2226}][Br]$	288[115]									
$[N_{4444}][Br]$	1862[115]		200[198]	178[198]					30[115]	
$[P_{4444}][Br]$	513[115]		407[198]	46[198]					9[115]	
$[P_{666(14)}][Br]$	2570[115]		708[198]							
$[P_{666(14)}][BF_4]$			2951[161]	3[198]						
$[P_{666(14)}][PF_6]$			>1995[166]							
$[P_{666(14)}][Tf_2N]$			>3020[198]	2[198]	79[166]					
$[P_{666(14)}][(CN)_2N]$			2512[161]							

Table 3.14. Toxicity (EC_{50}/mmol.L^{-1}) of functionalized ionic liquids toward different levels of biological complexity including enzyme (Acetyl Choline Esterase AChE), bacterium (*V. fischeri*), algae (*Scenedesmus vacuolatus* = *S. v*), duckweed (*L. minor*) and cell lines from rat (IPC 81).

R		Py-R	RmIm	RmMor	RmPip	RmPyr	N_{112R}
-C_2H_4-OH	IPC-81[53]	14.3[I]; 6.2[Tf_2N]	>20[I]; 5.8[Tf_2N]	>20[I]; 1.5[Tf_2N]	>20[I]; 4.5[Tf_2N]	>20[I]; 5.2[Tf_2N]	>20[I]; 6.3[Tf_2N]
	AChE[162]		0.913[halides]	0.919[halides]	0.221[halides]	0.43[halides]	
	V. f[120]		7.7[I]; 11.1[Tf_2N]				
	S.V[120]		>1[I]; 0.15[Tf_2N]				
	L.m[120]		1.65[I]; 1.65[Tf_2N]				
-CH_2CN	IPC-81[53]	6.1[Cl]; 3.2[Tf_2N]	>20[Cl]; 8[Tf_2N]	>20[Cl]; 3.4[Tf_2N]	>20[Cl]; 10[Tf_2N]	>20[Cl]; 6.4[Tf_2N]	>20[Cl]; 7.3[Tf_2N]
	AChE[162]		0.776[halides]	>1000[halides]	0.267[halides]	0.767[halides]	
	V. f[120]		>10[Cl]; 6.5[Tf_2N]				
	S.V[120]		>1[Cl]; 0.12 [Tf_2N]				
	L.m[120]		2.8[Tf_2N]				
-C_3H_6-OH	IPC-81[53]	>20[Cl]; 3.5[Tf_2N]	>20[Cl]; 4.6[Tf_2N]	>20[Cl]; 3.4[Tf_2N]	>20[Cl]; 4.2[Tf_2N]	>20[Cl]; 4.0[Tf_2N]	6.7[Tf_2N]
	AChE[162]		0.99[halides]	>1000[halides]	0.342[halides]	0.731[halides]	
	V. f[120]		>20[Cl]; 6.5[Tf_2N]				
	S.V[120]		>1[Cl]; 0.095 [Tf_2N]				
	L.m[120]		3.35[Cl]; 3.33[Tf_2N]				

R		Py-R	RmIm	RmMor	RmPip	RmPyr	N_{112R}
$-C_8H_{16}-OH$	IPC-81[200]		0.229[Br]				
	AChE[200]		0.019[Br]				
$-C_7H_{14}-COOH$	IPC-81[200]		>1[Br]				
	AChE[200]		>1[Br]				
$-CH_2-O-C_2H_5$	IPC-81[53]	2.1[Cl]; 1.3[Tf_2N]	4[Cl]; 1.6[Tf_2N]	3.3[Cl]; 2.3[Tf_2N]	17.2[Cl]; 2.6[Tf_2N]	0.85[Cl]; 1.8[Tf_2N]	3.9[Cl]; 6.3[Tf_2N]
	AChE[162]		0.407[halides]	0.92[halides]	0.139[halides]	0.072[halides]	
	V. f[120]		12[Cl]; 1[Tf_2N]				
	S.V[120]		0.89[Cl]; 0.09 [Tf_2N]				
	L.m[120]		>5[Cl];0.18[Tf_2N]				
$-C_2H_4-O-CH_3$	IPC-81[53]	>20[Cl]; 1.5[Tf_2N]	>20[Cl]; 1.8[Tf_2N]	>20[Cl]; 6.5[Tf_2N]	>20[Br]; 1.9[Tf_2N]	>20[Cl]; 2.0[Tf_2N]	>20[Cl]; 2.0[Tf_2N]
	AChE[162]		0.379[halides]	0.999[halides]	0.114[halides]	0.239[halides]	
	V. f[120]		15[Cl]; 0.67[Tf_2N]				
	S.V[120]		1.82[Cl]; 0.095 [Tf_2N]				
	L.m[120]		0.42[Cl]; 0.58[Tf_2N]				
$-C_2H_4-O-C_2H_5$	IPC-81[53]	17.5[Br]; 1.8[Tf_2N]	13.8[Br]; 1.5[Tf_2N]	>20[Br]; 4.9[Tf_2N]	>20[Br]; 2.2[Tf_2N]	>20[Br]; 1.6[Tf_2N]	>20[Cl]; 2.0[Tf_2N]
	AChE[162]		0.187[halides]	>1000[halides]	0.401[halides]	0.4[halides]	
	V. f[120]		19.2[Br]; 0.89[Tf_2N]				

R		Py-R	RmIm	RmMor	RmPip	RmPyr	N_{112R}
$-C_2H_4-O-C_2H_5$	*S.V*[120]		0.33[Bt]; 0.1[Tf_2N]				
	L.m[120]		0.3[Br]; 0.14[Tf_2N]				
$-C_3H_6-O-CH_3$	IPC-81[53]	>20[Cl]; 2.4[Tf_2N]	>20[Cl]; 2.2[Tf_2N]	>20[Cl]; 5.9[Tf_2N]	>20[Cl]; 1.9[Tf_2N]	>20[Cl]; 2.5[Tf_2N]	>20[Cl]; 6.7[Tf_2N]
	AChE[162]		0.405[halides]	>1000[halides]	0.158[halides]	0.545[halides]	
	V. f[120]		>20[Cl]; 1.6[Tf_2N]				
	S.V[120]		>1[Cl]; 0.115 [Tf_2N]				
	L.m[120]		0.98[Cl]; 0.55[Tf_2N]				
$-CH_2CH=CH_2$	Hela[166]	7.95[Cl]; 2.24[Tf_2N]	7.04 [Cl]; 1.63[Tf_2N]				31.25 [Br];
			7.85[BF_4]				2.91 [Tf_2N]

3.2 Biodegradability evaluation and biodegradation pathways of ionic liquids

Biodegradation is one of the main natural processes controlling the fate of organic chemicals in the environment.[201] All the living organisms, getting from microorganisms to plants and animals, can transform them either into degradation products (metabolites), that can present very different physico-chemical properties and biological activities than the parent molecule (more toxic, more persistent) or completely biodegrade them into carbon dioxide, water, mineral salts and biomass (ultimate biodegradation or mineralization), if the chemicals are biodegradable. This parameter (biodegradability) plays an important role in evaluating the environmental impact of the organic compounds studied. Biological processes have also gained interest due to their eco-friendly and cost-effective aspect.[201]

As microorganisms are very diverse and ubiquitous in the environment, most of the studies reported on ionic liquid biodegradability/biodegradation focused on this type of organisms. Several authors have attempted to define factors or rules on the molecular structure of the chemicals that can favor the mineralization of the compound or avoid its biodegradation, in order to predict its environmental behavior. Boethling *et al.*[202] carried out a pioneer work that was used for the design of biodegradable ionic liquids. He highlighted a number of factors that can improve the biodegradability:

- benzene rings that can be attacked by oxygenases, and unsubstituted linear alkyl chains

- groups that provide possible sites for enzymatic hydrolysis, especially oxygen atoms (in the form of hydroxyls, aldehydes, esters or carboxylic acids) but also amides.

The structural motifs generally increasing the resistance to aerobic biodegradation include:

- halogens, with chlorine and fluorine being particularly persistent

- chain branching, particularly where tertiary nitrogens or quaternary carbons are part of the structure, or where multiple branches are present in the same molecule

- nitro, nitroso, azo and arylamino groups

- polycyclic frameworks of the kind encountered in fused aromatic hydrocarbons
- heterocycles (e.g. pyridine rings)
- aliphatic ethers

These 'rules of thumb' are only guidelines and the presence of a single desirable or undesirable motif within a molecule does not guarantee either biodegradability or persistence in the environment.[2] In fact, the biodegradability of a chemical is affected not only by its structure but also by the exposure conditions. Fewson has summarized possible reasons for recalcitrance (resistance to microbial attack):[203]

1. Environmental reasons:

- Appropriate microorganisms do not exist or are not present;
- There are inadequate nutrients for the microbial population;
- The temperature, pH or pO_2 is too low or too high; ionic conditions are unsuitable;
- Concentration of substrate is too high (toxic) or too low;
- The substrate is not accessible to be attacked because it is too large and/or insoluble, or adsorbed or covalently attached to clays, humus, etc., or is physically inaccessible (not bioavailable).

2. Molecular reasons: the substrate

- is not transported into the cell;
- is not a substrate for the available enzymes;
- is not an inducer for the appropriate enzymes or transport systems.

Biodegradation studies of ionic liquids have been the subject or part of the subject of different recent reviews[2,106,107,108] that were used to build this bibliographic part.

3.2.1 Assays to determine the biodegradability of ionic liquids

In general, a first approach to assess biodegradability consists in the determination of parameters such as dissolved organic carbon (DOC), CO_2 production and/or oxygen consumption. Most of these assays correspond to normalized OECD test methods that permit the screening of ionic liquids for biodegradability in an aerobic aqueous medium or soil, along with their applicabilities (Table 3.15):

Table 3.15. Applicability of OECD test methods.[204]

Test	Analytical method	Inoculum	Ps	V	A
DOC Die-Away (301 A)	DOC	activated sludge (AS)	-	-	±
CO_2 evolution (301 B)	respirometry: CO_2 evolution		+	-	+
Closed bottle (301 D)	respirometry: dissolved oxygen	a secondary effluent from a domestic waste, water treatment plant or laboratory-scale unit	±	+	+
Modified OECD screening (301 E)	DOC		-	-	±
OECD 309	^{14}C labeling	river water	±	+	+
CO_2 headspace test (ISO 14593)	CO_2 evolution	activated sludge	+	+	+
ASTM 5988 (in soil)	CO_2 production/BOD		-	-	±

Suitable method to screen compound: +; unsuitable method to screen compound: -. Ps= Poorly soluble, V= Volatile, A= Adsorbing

Dissolved Organic Carbon Die-Away Test (OECD 301A)[204]

The chemical being evaluated is added to an aerobic aqueous medium inoculated with activated sludge microbial community after pre-conditioning (aeration at room temperature) and the evolution of CO_2 is measured regularly by combustion in a TOC apparatus and reported as a percentage of the theoretical maximum.

Modified Sturm test (OECD 301B)[204]

The chemical being evaluated is added to an aerobic aqueous medium inoculated with wastewater microorganisms and the evolution of CO_2 due to microbial respiration and the chemical degradation is measured by titration for a defined period of time in the dark (normally 28 day incubation period) and reported as a percentage of the theoretical maximum. Compounds which evolve more than 60% of the total CO_2 are considered to be a pass.

Closed bottle test (OECD 301D)[204]

The chemical being evaluated is added to an aerobic aqueous medium inoculated with

wastewater microorganisms and the depletion of dissolved molecular oxygen is measured for a defined period of time (normally 28 day incubation period) and reported as a percentage of the theoretical maximum (ratio of the Biological Oxygen Demand (BOD) to the Chemical Oxygen Demand (COD) both of them expressed as mg O_2 per mg compound). This value is corrected for the uptake of O_2 by the blank inoculum. Compounds which reach a biodegradation level higher than 60% are referred to as "readily biodegradable".

CO_2 Headspace test (ISO14593)[205]

This method allows the evaluation of the ultimate aerobic biodegradability of an organic compound in aqueous medium at a given concentration of microorganisms issued from activated sludge by analysis of inorganic carbon. Biodegradation was determined by measuring the net increase in total organic carbon (TOC) levels over time compared with unamended blanks in sealed bottles with an headspace of air which provides a reservoir of oxygen. The test ran for 28 days. The extent of biodegradation was expressed as a percentage of the theoretical amount of inorganic carbon (ThIC) based on the amount of test compound added initially.

Aerobic mineralization in surface water (OECD 309)[206]

This test measures the biodegradation of the chemical being evaluated over time at low concentration in aerobic natural surface water in the dark under aerobic conditions and agitation. Degradation is traced at appropriate time intervals by measuring either the residual ^{14}C or the residual concentration of the chemical studied.

CO_2 Monitoring of soil samples (ASTM 5988)[207]

The chemical being evaluated is mixed with soil sieved at 2-mm, humidified to about 90% of the WHC and adjusted at 15/1 for the C/N ratio, and placed in desiccators, sealed air-tight. The amount of CO_2 produced was determined by titrating 0.4 $mol.L^{-1}$ KOH solutions placed in the test and in the blank dessicators at regular intervals with 0.25 $mol.L^{-1}$ HCl to a phenolphthalein end-point. The extent of biodegradation was expressed as

a percentage of the theoretical amount of carbon dioxide based on the amount of test compound added initially.

Normally, the tests last for 28 days. Tests however may be ended before 28 days or be prolonged beyond 28 days (in particular in soils) depending on the speed of biodegradation.[189]

Many studies based on these biodegradability assays have been reported for ionic liquids, particularly the imidazolium family and are listed into table 3.16.

Table 3.16. Experimental studies on biodegradation of ionic liquids over standard 28 days.

Authors	Method	Ionic liquids	% biodegradation
Gathergood *et al.*[10]	301B	$[C_4mIm][PF_6]$; $[C_1COOC_2mIm] + [BF_4]$, $[Br]$	60%; 59%, 48%
	301D	$[C_1COOC_nmIm] + [Br]$, $[BF_4]$, $[PF_6]$, $[Tf_2N]$ (n = 1 - 8)	20 - 35%
		$[C_1CON(C_n)(C_{n'})mIm][Br]$ (n = 0 - 2; n' = 2, 4)	0%
Gathergood *et al.*[11]	301D ISO14593	$[(C_1COOC_{3\ or\ 5})C_{0\ or\ 1}mIm][Br]$	23 - 33% (301D)/ 24 - 41% (ISO)
		$[(C_1COOC_{3\ or\ 5})C_{0\ or\ 1}mIm][C_8SO_4]$	49 - 56% (301D)/ 61 - 67% (ISO)
Garcia *et al.*[12]	301D	$[C_4mIm]/[C_1COOC_3mIm] + [Br]$, $[Cl]$, $[BF_4]$, $[PF_6]$, $[Tf_2N]$, $[N(CN)_2]$	0% $[C_4mIm]$ / 30% $[C_1COOC_3mIm]$
		$[C_1COOC_3mIm][C_8SO_4]$	49%
Garbaczewska and Hupko[208]	309	$[C_4mIm] + [BF_4]$, $[PF_6]$; $[C_{1or4}Im][CC(OH)COO]$; $[C_1OC_4Im][CC(OH)COO]$	< 1%
		$[C_{10}Im][CC(OH)COO]$	100% (32 days)
Modelli *et al.*[17]	ASTM 5988 (in soil)	$[C_4mIm]/[C_2OC_1mIm] + [BF_4]$, $[N(CN)_2]$	17% / 0% (6 months)
Dai *et al.*[209]	301D (PB)	$[C_6mIm][Br]$ / $[C_8mIm][PF_6]$	AS: 18% / 21%; acclimatized AS: 25% / 62%
Morrissey *et al.*[19]	ISO14593	$[C_1COOC_5mIm][C_8SO_4]$, $[C_1COOC_2OC_{3or4}mIm][C_8SO_4]$ $[C_1COOC_2OC_2OC_{3or4}mIm][C_8SO_4]$, $[C_1COOC_2OC_4mmIm][C_8SO_4]$	> 60 %
		$[C_1COOC_4mIm][C_8SO_4]$,$[C_1COOC_2OCmIm][C_8SO_4]$, $[C_1COOC_2OC_2OC_{1or2}mIm][C_8SO_4]$, $[C_1COOC_2OC_{1or2}mIm][C_8SO_4]$	55 - 59 %
		$[(C_1C{=}OPyr)mIm][C_8SO_4]$,$[(C_1C{=}O(1\text{-}Pyr))mmIm][C_8SO_4]$, $[C_1C{=}ON(C_2OC)_2mIm][C_8SO_4]$	25 - 35 %

Authors	Method	Ionic liquids	% biodegradation[a]
Wells and Coombe[138]	%oxygen uptake	$[C_4mIm]$ + $[Cl]$, $[PF_6]$; $[C_4Py][Cl]$, $[P_{2244}][(EtO)_2PO_2]$, $[N_{1188}][Tf_2N]$	0 %
Stolte *et al.*[210]	301D (PB)	$[C_nmIm][Cl]$ (n=2,4,6); $[C_{2or3}OHmIm][Cl]$; $[C_1C_6H_6mIm][Cl]$; $[C_1CNmIm][Cl]$; $[C_1OC_2mIm][Cl]$; $[C_2OC_1mIm][Cl]$; $[C_2OC_2mIm][Br]$; $[C_3OC_1mIm][Br]$; $[C_2OC_1mIm][Cl]$; $[C_{2or4}Py][Cl]$; $[C_4mmPy][Cl]$; $[C_2(NCC)Py][Br]$; $[C_4(NCC)Py][Cl]$	0 %
		$[C_8mIm][Cl]$; $[C_8mPy][Cl]$; $[C_8Py][Cl]$; $[C_6(NCC)Py][Cl]$; $[C_8OHmIm][Cl]$; $[C_7COOHmIm][Cl]$;	100 %
Docherty *et al.*[14]	Modified 301A	$[C_nmIm][Br]$/ $[C_nmPy][Br]$ (n =4,6,8)	0 %(n = 4) 54 % / 97% (n = 6) 41 % / 96 % (n = 8)
Harjani *et al.*[211]	ISO14593	$[C_4mIm][C_nSO_4]$ (n = 6 - 12)	34 % (n =6) - 58 % (n = 12)
		$[C_6mIm][C_nSO_4]$ (n = 6 - 12)	30 % (n =6) - 49 % (n = 12)
		$[C_2OHmIm][Br]$, C_4SO_3mIm, $[C_1C_6H_6mIm][Br]$, $[C_1C{=}CmIm][Br]$, $[C{=}CbIm][I]$, $[C_4mIm][(C_4O)_2PO_2]$, $[C_4mIm][CC(OH)COO]$	0 %
		$[C_4OOC_1mIm][I]$, $[C_1C_6H_6mIm][C_8SO_4]$,$[C_4mIm][saccharinates]$, $[C_1SO_3]$	5 - 25%
		$[C_4OOCCmIm][C_8SO_4]$, $[C{=}CbIm][C_8SO_4]$, $[C_1C{=}CmIm][C_8SO_4]$	30 - 49%
		$[C_1COOC_5mIm][saccharinates]$, $[C_1COOC_5mIm][C_{12}SO_4]$	> 60 %
Harjani *et al.*[212]	ISO14593	$[C_1COOC_2Py]$ + $[Br]$, $[C_8SO_4]$; $[m(COOC_4)Py][Tf_2N]$, $[(m\ or\ b)(COOC_4)Py][C_8SO_4]$	70 - 89%
		$[b(C{=}ONC_4)Py][C_8SO_4]$	30%
Harjani *et al.*[13]	ISO14593	$[C_4Py]$ + $[Br]$, $[Tf_2N]$, $[PF_6]$; $[C_4mPy][Br]$, $[C_{10\ or\ 16}Py][Br]$	0 - 10%
		$[C_4Py][C_8SO_4]$, $[C_4mPy][C_8SO_4]$	37%, 40%
		$[C_1COOC_2Py]$ + $[PF_6]$, $[Tf_2N]$; $[m(COOC_4)Py]$ + $[I]$, $[C_8SO_4]$, $[PF_6]$	> 70%

Authors	Method	Ionic liquids	% biodegradation[a]
Docherty *et al.*[141]	301A (RP-HPLC/MS)	$[C_nmPy][Br]$ (n = 4,6,8)	n =4, 0%; n =6, 28%; n =8, 90%
Stasiewicz *et al.*[163]	301D	$[(C_1OC_n)(OH)Py]$ + saccharinates / acesulfamates / [Cl]	43% / 24% / 40% (n = 3) 13% / 22% / - (n = 4) 31% / 39% / - (n = 6) 32% / 41% / 44% (n = 7) 72%/ 49%/48% (n = 11) 20%/32%/25%/ (n = 18)
Pham *et al.*[107]	Modified 301E (PB)	$[C_4mPy][Br]$	> 60 %
Ford *et al.*[213]	ISO 14593	$[C_2OHPy][Br]$_$[Tf_2N]$ / $[(C_2OH)(COOC_4)Py][Tf_2N]$ $[(C_4)(C_2OC_2)Py][C_8SO_4]$_$[Tf_2N]$ / $[(C_2OH)(C_1OCPy][Tf_2N]$ $[(C_n)(C_1OC)Py][C_8SO_4]$ (n =4 or 8) $[(C_1COOC_{n'})(C_1OC)Py][C_8SO_4]$ (n'=2 or 5)	65%_62% / 71% 36%_1% / 6% 32% (n = 4), 31% (n = 8) 47%(n'= 2), 51% (n'= 5)

For the same ionic liquid, the biodegradability data obtained from the different tests are not always the same, and sometimes are rather disperse. These respirometric tests present several limits: (i) the nature and quantity of the inoculum play an important role in biodegradability assessment. For example, cell densities in these tests vary considerably (Closed Bottle test: 10^1 - 10^3 cells/mL; Modified Sturm test: 10^4 - 10^6 cells/mL);[10] (ii) the activated sludge sample taken as inoculum can greatly vary in microbial diversity according to the wastewater treatment plant chosen and even in the same plant according to the season or to the influent content[141]; (iii) the toxicity of the ionic liquids and/or their biodegradation products can have a negative impact on the growth of microorganisms able to degrade them[214]; (iv) the microbial respiration may be affected by the presence of the chemical or its degradation products and the blank or negative control is not therefore a real blank. Despite the results of the studies are not always constant, tendencies can still be found.

A. Imidazolium-based ionic liquids

Most of the studies deal with 1-alkyl-3-methylimidazolium-based ionic liquids. The ones containing a short alkyl side chain (less than 5 carbon atoms, < C_5), even when unsaturated groups (allyl or vinyl) or a sulfate group is incorporated,[211] show low biodegradability. In many cases, longer alkyl chain (> C_6) on the cation leads to a better biodegradability.[14,210,211] Extended incubation times (up to 49 days) show partial mineralization of the imidazolium-based ionic liquids containing longer alkyl chain ([C_6mIm] and [C_8mIm]).[14] Even [C_{10}mIm] was found to be completely undetectable in the sample after 13 day of incubation in the study of Garbaczewska and Hupko.[208] The improvement of the biodegradability is possibly due to the increased lipophilicity of these ionic liquids, which would lead to faster absorption of compounds through the cell membranes of the microorganisms present in the test.[210] In the study of Harjani *et al.*[211], the biodegradation rate of [C_4mIm][$C_n SO_4$] ionic liquids were slightly higher than [C_6mIm][$C_n SO_4$], which may be merely attributed to the larger ratio of mineralized organic carbon (anion) to the total organic carbon content (anion + cation). Introduction of an additional methyl group in the C2 position of the imidazolium ring showed no

improvement of biodegradability compared to the C2-unsubsitituted ionic liquids.[19] In all these cases, the imidazolium ring does not undergo biodegradation over the period of the tests.

The effect of the anion on biodegradability was also examined on $[C_4mIm]^+$ cation combined with different anions $[BF_4]^-$, $[PF_6]^-$, $[Br]^-$, $[N(CN)_2]^-$, $[Tf_2N]^-$. No compound show significant biodegradability except the one with the anion $[C_8SO_4]^-$.[215]

As previously studied, the ionic liquids with longer alkyl chain exhibit higher toxicities, hence the long alkyl chain is not an ideal option and the researchers focus on improving biodegradability by incorporation of functional groups in the side chain of cation.

The introduction of an ester group in the side alkyl chain of imidazolium-based ionic liquids enhanced biodegradabilities compared with the corresponding non-functionalized ones.[10,11,19,211] The presence of an ester moiety provides a site for possible enzymatic cleavage leading to the parent imidazolium, which has been shown to be recalcitrant to biodegradation[209,210,211] and the corresponding primary alcohol. Esters with an alkyl chain > 4 carbons proved to be the more biodegradable. In this series, the biodegradability slightly depends on the anions.[12] In Gathergood *et al.*'s study, the biodegradation efficiency decreases in the order $[PF_6]^-$ (60%) > $[BF_4]^-$ (59%) > $[Br]^-$ (48%) >> $[Tf_2N]^-$ > $[N(CN_2)]^-$.[215] An increase of the alkyl chains length in alkyl sulfate anions enhances also the biodegradability of ionic liquids and $[C_{8\text{-}12}SO_4]^-$[10,19,211] gave better biodegradation than other anions due to their high propensity to degrade. Other anions have also shown good mineralization abilities such as lactate and saccharinate.[211] The combination of an ester functionality in the alkyl chain of the imidazolium cation and of octylsulfate as anion gave the first "readily biodegradable" compounds $[(C_1COOC_{3\text{ or }5})C_{0\text{ or }1}mIm][C_8SO_4]$ identified by the CO_2 Headspace test.[11]

Other functional groups have been introduced into the side alkyl chain of the imidazolium cations to improve their biodegradability. The incorporation of a terminal hydroxy group into short alkyl chain, a phenyl ring, an anionic sulfonate group, an allyl, a vinyl group,[211] amide groups[10] does not show obvious effect on biodegradability.

Nevertheless the insertion of terminal hydroxy groups into long chained ionic liquids might be advantageous.[210]

Another oxygenated group that has been widely tested is ether or polyether function in the presence or absence of ester. Whilst ionic liquids with a butoxy or propoxy- terminus are readily biodegradable, those with methoxy- or ethoxy- terminal substitution remained slightly below the 60% threshold for readily biodegradability.[19] Morrissey *et al.* have found that the addition of one or two ether functions into the ester chain does not have a detrimental effect on the biodegradation compared to the alkyl ester analogues.[19] $[C_1OC_4Im][CC(OH)COO]$ (concentration decreases from 14% to 10% over 77 days) displays slightly higher biodegradation than $[C_4Im][CC(OH)COO]$ (23% to 19%), but the improvement is either caused by the increase of the chain length or by the inclusion of ether group.[208]

In the study of ionic liquid biodegradation in soil, Modelli *et al.*[17] have shown that the presence of an ether group in the side chain decreased its biodegradation. $[C_4mIm]^+$ based ionic liquids ($[BF_4]^-$ 52.1 ± 6.6%, $[N(CN)_2]^-$ 17.0 ± 4.2% after six months) were more biodegradable than $[C_2OC_1mIm]^+$ (< 4%) for the same period.[17]

B. Pyridinium-based ionic liquids

The pyridinium-based ionic liquids containing alkyl chain up to C_4 are not biodegradable, with no discernable effect of the anions.[13,14,138,163,210] For longer alkyl chain, the results obtained are very dependent on the studies even for similar ionic liquid concentrations and the variability of the tests are evidenced. The $[C_4Py]^+$ pyridinium ionic liquid tested by Docherty *et al*. was either not biodegradable[141] or fully mineralized in an extended incubation time (41 days).[141] The modest increase of alkyl chains (C_6 and C_8) enhanced the biodegradability[14,210] and $[C_8mPy]^+$ can even reach the threshold for readily biodegradability.[14,141,210] The monitoring of DOC showed that the limiting step is the cleavage of the pyridinium ring. Once the ring has been opened, mineralization occurred rapidly. The pyridinium nucleus is therefore not refractory to biodegradation.[141] Conversely, a further increase of the alkyl chain (C_{10} – C_{16}) avoids the biodegradation[13].

The influence of anions on biodegradability is not significant.[13,163] The $[C_8SO_4]^-$ anion seems to have only a meagre influence on enhancing the biodegradability. The only negative effect is observed with $[Tf_2N]^-$ in the series of $[C_4Py]^+$ based ionic liquids: this anion offers slightly lower biodegradability than $[Br]^-$, $[C_8SO_4]^-$ and $[PF_6]^-$. This is mostly due to its lower bioavailability caused by its lower solubility in water.[13] The presence of the methyl group at the C3 or C3 together with the C5 position of the pyridine ring does not help improving the biodegradability.[13]

The introduction of an ester group seems to have a profound effect on the biodegradability of ionic liquids.[13,210] Ionic liquids with a pyridinium cation bearing an ester group containing substituent at position 1 or 3 show excellent biodegradation and are "readily biodegradable".[212,213]

The derivatisation of the pyridinium cycle with amide functions,[212] carbamate and acetal linkage with another pyridinium ring[213] does not increase the biodegradability. The introduction of en ether group was also tested and brought lower positive effect than ester[213], even though Stasiewicz *et al.*[163] prepared a "readily biodegradable" based on 1-alkoxymethyl-3-hydroxypyridinium and saccharinate $[(C_1OC_{11})(OH)Py]$. In this series, the biodegradability is not positively correlated with the alkoxy chain length: (n = 11) > (n = 3) > (n = 6 and 7) > (n = 18) > (n = 4).[163]

The 4-(dimethylamino)pyridinium head group $[C_n(NCC)Py]^+$ is highly lipophilic[53], and when n = 6, the corresponding ionic liquid is completely degraded within 31 days but no biological breakdown could be observed for (n = 2 and 4).[210]

C. Conclusions

In conclusion, pyridinium-based ionic liquids are more biodegradable than imidazolium ones. The length of the alkyl side chain on the cation has a great influence on biodegradability and only the C_6 to C_8 carbon chains can be degradable. The introduction of an ester function increases dramatically the biodegradability of the ionic liquids due to their potential enzymatic cleavage by esterases. The anion can also play a role on this parameter: alkylsulfates have been shown to be easily biodegradable and therefore increase the whole biodegradation of the ionic liquids.

Nevertheless the normalized OECD assays used give very variable results between them. The same ionic liquid can be declared "readily biodegradable" with one test and not with another one. Moreover, these tests do not give any information on the degradation compounds formed that can be more toxic than the parent molecule.

To get further and obtain more detailed information, a few studies have monitored the biodegradation kinetic of the ionic liquid itself with pure strain or mixed microbial community or have even tried to determine the metabolic pathway of these compounds using different analytical tools such as NMR or LC-MS/ LC-MS-MS.

3.2.2 Biodegradation pathways of ionic liquids

Only a few studies focused on the establishment of biodegradation pathways of ionic liquids by wastewater activated sludge or pure isolated strain under aerobic and anaerobic conditions. Most of these studies concern alkyl imidazolium and pyridinium-based ionic liquids.

A. Imidazolium-based ionic liquids

For short alkyl side chain $[C_4mIm]^+$, no biodegradation was observed with different microbial communities.[14] Nevertheless, a pure fungal strain able to use this ionic liquid as the sole source of carbon was isolated by Esquivel-Viveros *et al.*[216] This *Fusarium* sp. is able to degrade 80% of $[C_4mIm][PF_6]$ at high concentration (21 $g.L^{-1}$) in a membrane-aerated biofilm reactor within 28 days of incubation. No metabolite was identified.

Longer alkyl chain imidazolium-based ionic liquids $[C_6mIm]^+$ and $[C_8mIm]^+$ have been shown to be biodegradable. The ^1H NMR monitoring of $[C_6mIm]^+$samples taken at T0 and after 39 days of incubation showed a partial degradation of the side alkyl chain but the imidazolium ring remains intact.[14] The metabolic pathways of $[C_8mIm][Cl]$ and $[C_8OHmIm][Br]$ were studied in details under both aerobic[14,210] and anaerobic[200] conditions. The anaerobic biodegradation of $[C_8mIm][Cl]$ is not initialized but the presence of a terminal hydroxy group lead to the biodegradation of the side chain. A residual rest of $[C_3OHmIm]^+$ was still detected even after 10 months.[200] A similar behavior has been

observed under aerobic conditions, except that the initial step of terminal hydroxylation of the alkyl side chain in [C_8mIm][Cl] can occur due to the presence of molecular oxygen and monooxygenase. This degradation product can undergo ω-oxidation and the carboxylic acid formed is submitted to β-oxidation[14,210] (Figure 3.1).

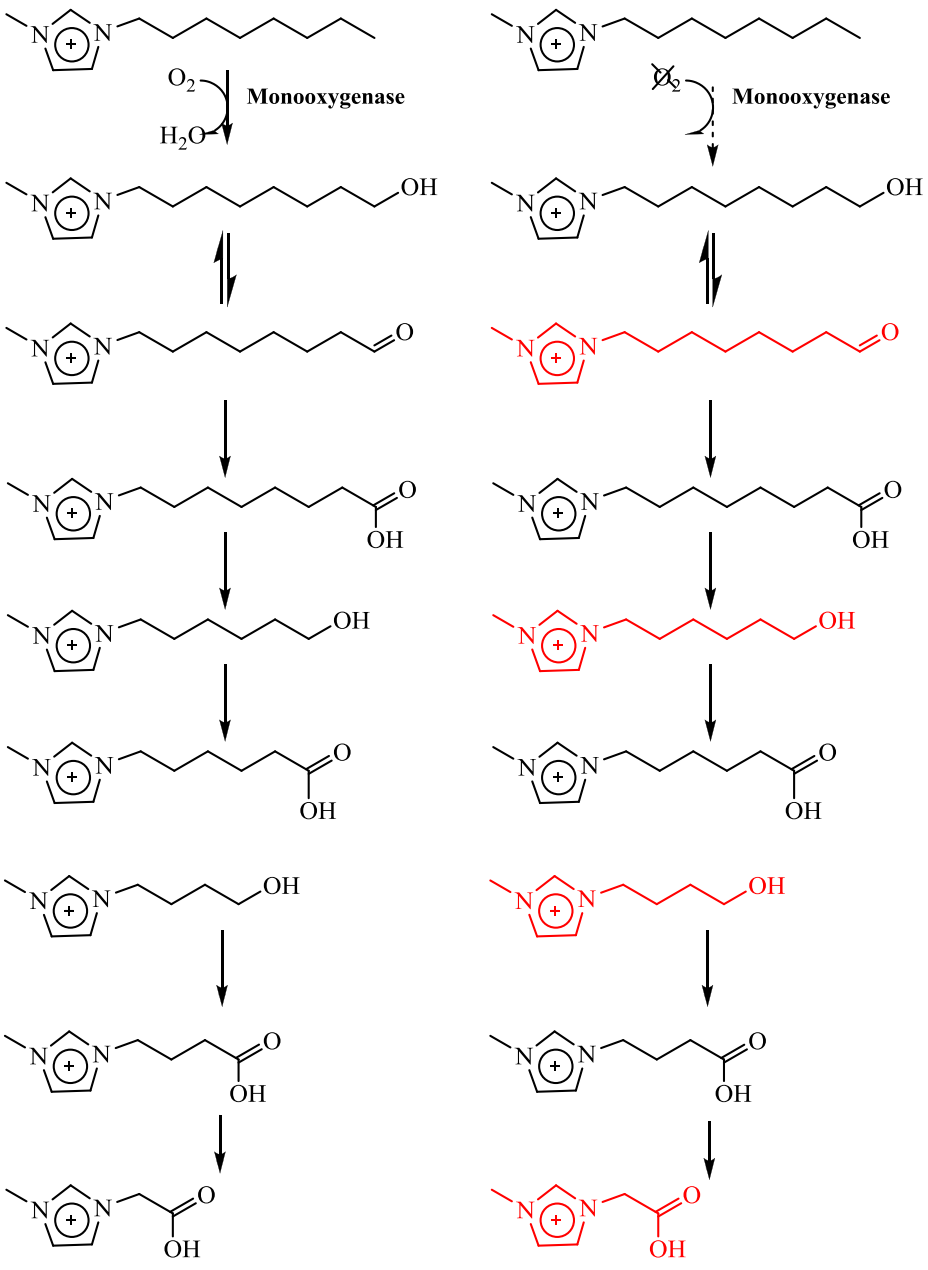

Figure 3.1. Proposed aerobic[210] and anaerobic[200] metabolic pathways for [C_8mIm][Cl] and [C_8OHmIm][Br]. The chemical structures in black were found by MS measurements and the ones in red are theoretical intermediates.

This degradation pathway is in complete agreement with the theoretical studies carried out on $[C_4mIm]^+$ by Stepnowski and Storoniak[89] and Jastorff *et al.*[112] that have based their work on the involvement of monooxygenases such as cytochromes P450 as key enzymes (Figure 3.2.).

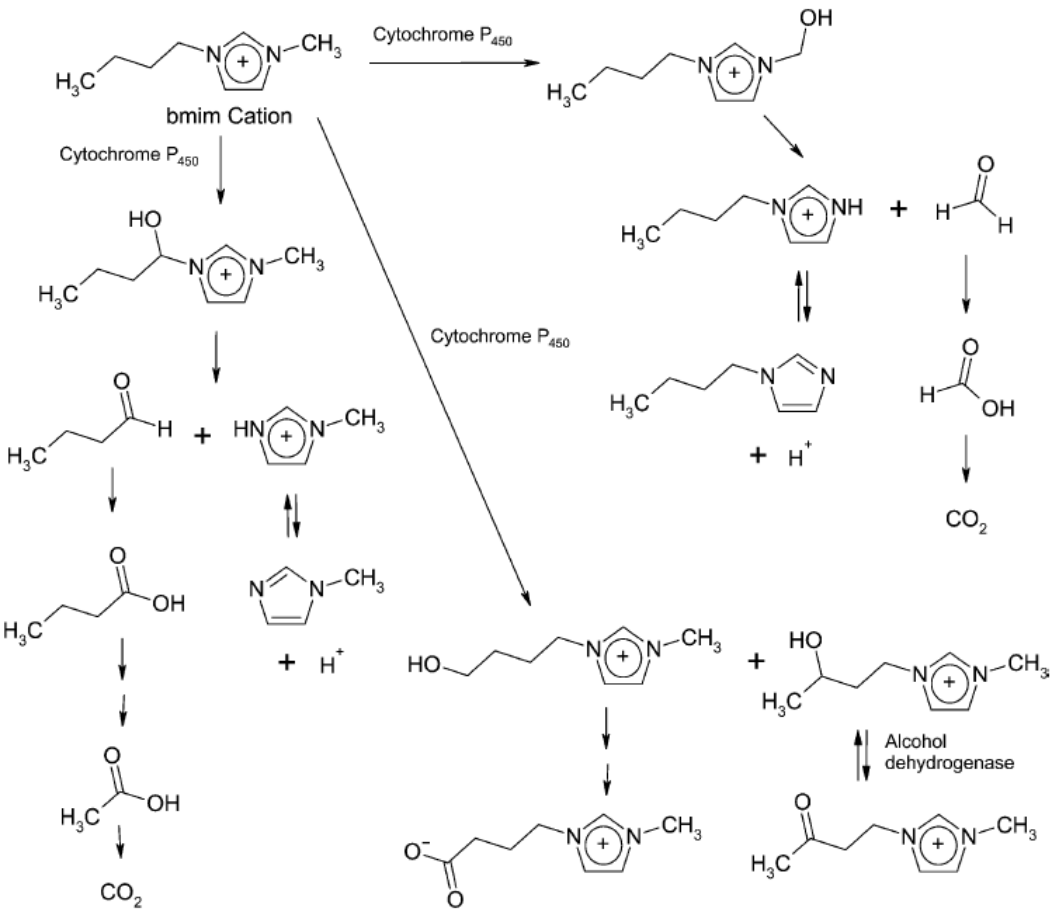

Figure 3.2. Theoretically predicted metabolism scheme for $[C_4mIm]$.[112]

Other pathways have been evidenced: hydroxylations in another position of the alkyl chain than the terminal one[210] or opening of the imidazolium ring in the case of acclimatized activated sludge.[209] In this last study[209], the biodegradation rate of $[C_8mIm][PF_6]$ was less than 20% when the inoculum was original activated sludge while biodegradation ratio reached 60% when the inoculum was acclimatized activated sludge (Figure 3.3).[209]

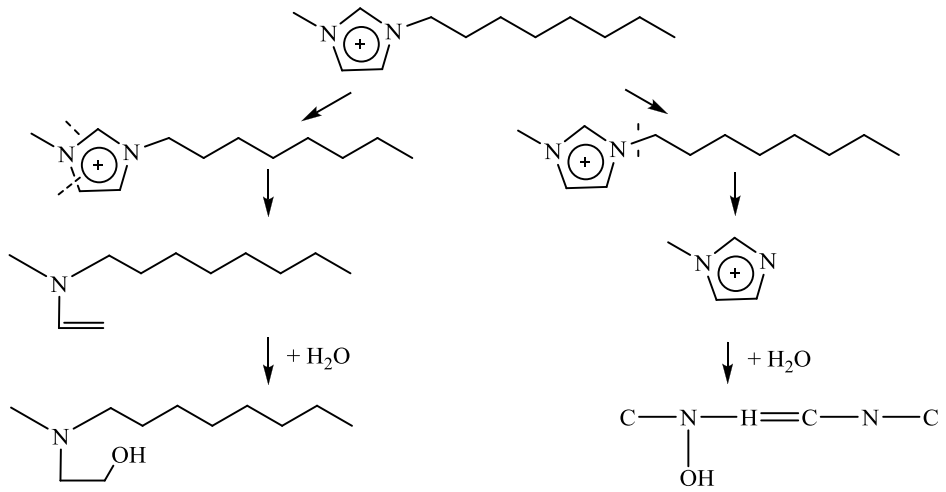

Figure 3.3. Pathways of biodegradation of $[C_8mIm][PF_6]$ by acclimatized activated sludge.[209]

B. Pyridinium-based ionic liquids

Pyridinium-based ionic liquids even with short alkylchain have been shown to be readily biodegradable.[14] Zhang *et al*. have found two strains able to use $[C_2Py][BF_4]$ as the sole carbon- and nitrogen-sources: *Corynebacterium* sp. issued from an axenic culture of soil[217] and *Pseudomonas fluorescens* ATCC 55241.[134] These two strains have different metabolic pathways: *Corynebacterium sp.* cleaved the pyridinium ring between C2 and C3 leading to low molecular organic acid degradation products that can be assimilated by the microorganism (Figure 3.4[217]); *P. fluorescens* might degrade N-ethylpyridinium cation by oxidation of its side chain leading to pyridinium as the final product. A similar process of pyridine ring cleavage was recorded in the biotransformation of pyridine by *Bacillus* strain.[218]

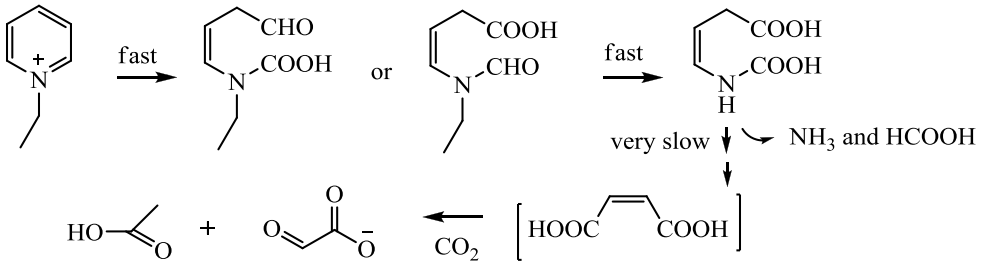

Figure 3.4. Biodegradation pathways of $[C_2Py]^+$ by an axenic culture of soil (*Corynebacterium sp*.)[217]

In contrast to the results obtained from the measure of DOC[210] and CO_2[13], [C_4mPy][Br] was found to be metabolized by the activated sludge microbial community. Pham *et al.*[107] have proposed two possible pathways of biodegradation for [C_4mPy][Br] according to LC-MS analyses, corresponding to sequential oxidation steps at different positions of the alkyl side chains but no cleavage of pyridinium ring was observed (Figure 3.5).

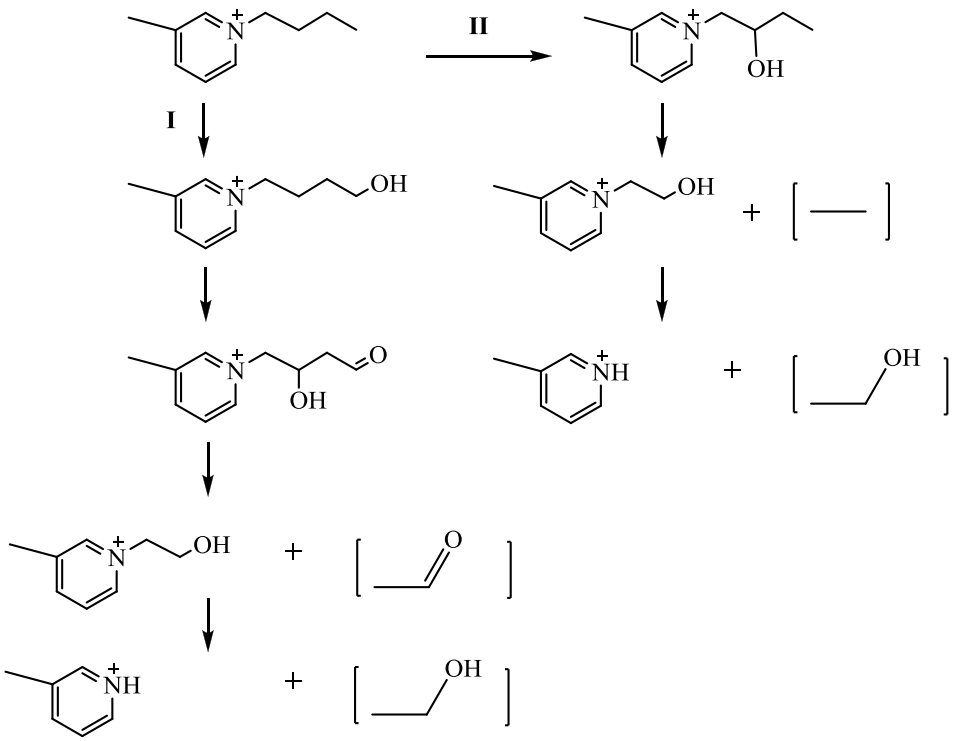

Figure 3.5. Biodegradation pathways of [C_4Py][Br] by microorganisms of activated sludge. The intermediates shown in brackets were not detected or confirmed.[107]

Another detailed study using RP-HPLC/MS and MS/MS suggests that fundamentally different biodegradation pathways occur depending on the length of the alkyl chain.[141] $[C_8mPy]^+$ is readily biodegradable through initial hydroxylation of the side alkyl chain and then β-oxidation. For $[C_6mPy]^+$, degradation by hydroxylation occurs separately on the side chain and on the aromatic ring as well as by unsaturation of the hexyl chain; $[C_4mPy]^+$ is more resistant to biodegradation because initial hydroxylation occurs on the more recalcitrant pyridinium ring instead of the short alkyl chain. The biodegradation involves also unsaturation of the butyl chain.

C. Ionic liquids with other cations

The biodegradation of ionic liquids with other head groups is rarely studied.

In Wells and Coombe's study[138], $[P_{2244}][(EtO)_2PO_2]$ presents only 9% of biodegradation with the BOD measurement. The biodegradability of morpholinium and piperidinium-based ionic liquids have not been investigated so far, but their N-alkylation probably will lead to poorly biodegradable compounds.[210]

Ammonium-based ionic liquids. Choline cation ($[N_{1112OH}]^+$) has been previously reported to undergo almost complete biodegradation (93%) under aerobic conditions.[202] In Yu *et al.*'s study[15], the choline based ionic liquids associated with naphthenic acid derivatives anions are more biodegradable than their counter-parts and almost all the tested ionic liquids passed readily biodegradation level except for 2-naphthoxyacetate and anthracene-9-carboxylate anions. This low biodegradability of the ionic liquids with these two anions is in accordance with Boethling's rule of thumb that polycyclic aromatic hydrocarbons can lead to an increase in resistance to biological breakdown[202], but both of the four- non aromatic ring anions passed.

Choline cation was only partially degraded by fungal isolate *P. corylophilum*.[131] Amongst the linear chain anions $[C_nCOO]^-$, n = (4, 5, 6, 8) completely disappeared and n = (2, 3) were only partially degraded. The branched chain anions, 2,2-dimethylpropanoate and 2-methylpropanoate were more resistant to fungal attack, leading to null or partial degradation, which is in accordance with Boethling's rule of thumb.[202]

The actual results on other ionic liquids are too limited to achieve some conclusions and more researches are needed. In this thesis, the biodegradabilities of ionic liquids with pyrrolidinium and other ammonium head groups in presence of pure bacterial species or activated sludge were studied.

D. Conclusion

In conclusion, studies on the biodegradation of ionic liquids are still at an early stage, and only imidazolium and pyridinium-based ionic liquids have been studied in some details. The imidazolium ring is resistant to biodegradation and the modification of the side chain on cation (introduction of an ester group or of a terminal hydroxy group) and the

selection of the anion (alkylsulfate) can improve significantly their biodegradability. Pyridinium-based ionic liquids show a generally higher biodegradability than imidazolium salts. The pyridinium ring can be degraded if the side chain on cation is moderately tuned, *i.e*. inclusion of functional groups in the side chains of cations. Studies on other cations are needed.

The detailed studies of biodegradation pathways for ionic liquids are rare. The identification of degradation products that could exhibit more hazardous risks is of first important.

In order to obtain ‘‘fully green’’ ionic liquids, the starting materials must be at least non-toxic, whilst for a perfect solution, they should be renewable. Moreover, development of new ‘‘green’’ ionic liquids still requires relatively low cost and easy preparation. Biorenewable natural compounds become ideal candidates from the point of view of both environmental and economical concerns.[219,220] A few successful samples have been reported, for example, using lactates,[22,123,221] sugar substitutes[222,223] and amino acids[224] to prepare anions, and sugars[225] and amino acids[226] to prepare cations.

4. Collection of papers

FULL PAPERS

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The Presence of Functional Groups Key for Biodegradation in Ionic Liquids: Effect on Gas Solubility

Yun Deng,[a] Saibh Morrissey,[b] Nicholas Gathergood,[b] Anne-Marie Delort,[c] Pascale Husson,[a] and Margarida F. Costa Gomes*[a]

The effect of the incorporation of either ester or ester and ether functions into the side chain of an 1-alkyl-3-methylimidazolium cation on the physico-chemical properties of ionic liquids containing bis(trifluoromethylsulfonyl)imide or octylsulfate anions is studied. It is believed that the introduction of an ester function into the cation of the ionic liquids greatly increases their biodegradability. The density of three such ionic liquids is measured as a function of temperature, and the solubility of four gases—carbon dioxide, ethane, methane, and hydrogen—is determined between 303 K and 343 K and at pressures close to atmospheric level. Carbon dioxide is the most soluble gas, followed by ethane and methane; the mole fraction solubilities vary from 1.8×10^{-3} to 3.7×10^{-2}. These solubilities are of the same order of magnitude as those determined for alkylimidazolium-based ionic liquids. The chemical modification of the alkyl side chain does not result in a significant change of the solvation properties of the ionic liquid. All of the solubilities decrease with increasing temperature, corresponding to an exothermal solvation process. From the variation of this property with temperature, the thermodynamic functions of solvation (Gibbs energy, enthalpy, and entropy) are calculated and provide information about the solute–solvent interactions and the molecular structure of the solutions.

Introduction

The negligible vapor pressure of ionic liquids minimizes an important exposure pathway for humans and the environment. The combination of this nonvolatility with their thermal stability and nonflammability makes them promising candidates for green solvents. However, an "ideal" green solvent should also be nontoxic and not persist in the environment. The large-scale development of ionic liquids is thus intimately linked to the assessment of their degradability and toxicity in the environment; the ideal ionic liquid presenting both a reduced toxicity and an improved biodegradability.

Ionic liquids are considered as designer solvents because their properties can be changed by either the appropriate cation–anion combination or by chemically modifying their organic side chains. Their properties can thus be adjusted for particular applications and new ionic liquids, generically denominated as "task-specific ionic liquids",[1,2] may be synthesized. The pioneering works of Gathergood and Scammells[3–6] made use of this concept and showed that the introduction of ester groups, which are susceptible to enzymatic hydrolysis, into the alkyl side chains of methylimidazolium based ionic liquid greatly improves the degradability of these compounds.

Alkylimidazolium-based ionic liquids are more readily biodegradable when the length of the alkyl chain on the imidazolium ring increases,[7–9] but simultaneously Wells and Coombe[10] have shown that imidazolium-, pyridinium-, phosphonium-, or ammonium-based ionic liquids can present high levels of toxicity towards the microorganisms responsible for their biodegradation in the environment. Docherty et al.[9] have observed complete mineralization of hexylmethylpyridinium bromide, and considered the ionic liquid octylmethylpyridinium bromide as readily biodegradable within criteria established by the organization for economic co-operation and development (OECD).[11] Thus, a rational molecular design of ionic liquids seems necessary to improve their biodegradability and toxicity characteristics.

Gathergood and Scammells[3–5] based the design of their ionic liquids on the "rules of thumb" for improving biodegradative properties of surfactants by Boethling[12] and the design of "soft" antimicrobials by Bodor et al.,[13] and applied them to the synthesis of new cations. The nature of the anion is also fundamental for the global degradability of ionic liquids. With the introduction of an *n*-octyl sulfate anion,[14] the first ionic liquid that can be classified as "readily biodegradable" under aerobic conditions was prepared: 3-methyl-1-(pentoxycarbonylmethyl)imidazolium octyl sulfate. Gathergood et al. have demonstrated that the inclusion of ether groups into the side chain of imidazolium-based ionic liquids also leads to readily

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biodegradable ionic liquids[15] and significantly reduces the toxicity compared with alkyl ester derivatives. Harjani et al.[16,17] and Stasiewicz et al.[18] also observed an increased biodegradability of pyridinium-based ionic liquids by the inclusion of oxygen-containing polar groups (ester side chain moiety,[16,17] alcohols,[18] ether groups[18]) in the cation or by changing the anion of the ionic liquid (acesulfamates, saccharinates).[18] Ionic liquids composed of choline cation[19] (combined with propionate, triglate, maleate, or succinate) and naphtalenic acid ionic liquids[20] are also attractive candidates.

The chemical modifications introduced to increase the biodegradability will surely modify the chemical reactivity and the physicochemical properties of these compounds. Indeed, even subtle changes in the structure of an ionic liquid can dramatically affect its physical properties. It is of prime importance to correctly characterize the modified ionic liquid if it is to be used in chemical processes.[21]

The objective of the work is to study the influence of the introduction of ester or ester and ether groups into the alkyl side chains of the imidazolium ring of the cation and of two different anions [bis(trifluoromethylsulfonyl)imide and octyl sulfate] on different physico-chemical properties of the ionic liquids, in particular on their densities and on their capability of dissolving different families of gaseous solutes commonly used in reaction mixtures. This information is useful for the calculation of vapor/liquid equilibria in reaction systems or separation processes.

Four gaseous solutes were considered: methane, ethane, hydrogen, and carbon dioxide. Their solubility in four ionic liquids (Table 1), of which the alkyl side chains were functionalized with ester or ester and ether functions, was measured as a function of temperature at a pressure close to atmospheric level using a high-precision isochoric saturation method. The Henry's law constants and thermodynamic functions were calculated from these experimental solubilities. In addition to these properties, the densities of the pure ionic liquids were measured as a function of temperature.

Results and Discussion

Density

The densities at 293 K of the four ionic liquids considered varied from 1.14 to 1.48 g cm^{-3}, with a slight decrease with temperature (from 1.11 to 1.43 g cm^{-3} at 353 K). As already observed in the literature,[22] the densities are related to the masses of the ions: $[C_1COOC_5mIm][C_8SO_4]$, which has the smallest mass, exhibits the lowest densities, while the Tf_2N-based ionic liquids have densities greater than 1.34 g cm^{-3}. The density of the octylsulfate-based ionic liquid is comparable to that of 1-butyl-3-methyl imidazolium octylsulfate, $[C_4mIm][OcSO_4]$ (1.06 g cm^{-3} at 313 K).[23,24] Concerning the three Tf_2N-based ionic liquids, no influence of the chain length or the addition of ether groups was observed. Their densities were similar to the density of 1-hexyl-3-methyl imidazolium bis(trifluoromethylsulfonyl)imide $[C_6mIm][Tf_2N]$ (1.37 g cm^{-3} at 303 K).[25] The logarithm of the densities was fitted as a linear function of temperature:

$$\ln(\rho) = a + bT \quad (1)$$

The parameters a and b of Equation (1) are presented in Table 2.

Table 2. Parameters a and b of Equation (4), and the average absolute deviation (AAD) from the experimental data.

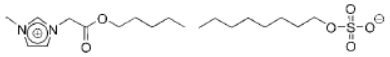
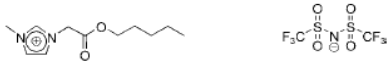
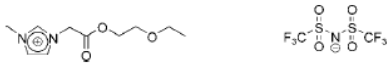
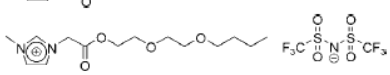
Ionic liquid	a	b	AAD
$[C_1COOC_5mIm][C_8SO_4]$	0.3259	-6.33×10^{-4}	0.04 %
$[C_1COOC_5mIm][Tf_2N]$	0.5478	-6.76×10^{-4}	0.13 %
$[C_1COOC_2OC_2mIm][Tf_2N]$	0.5997	-6.90×10^{-4}	0.01 %
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	0.5339	-6.93×10^{-4}	0.02 %

Group contribution models have been developed for the prediction of the volumetric properties of ionic liquids.[25–27] Using this tool, we have calculated the densities of the functionalized ionic liquids studied in the present work. Canongia Lopes et al.[28] have observed that the molar volume of an ionic liquid can be calculated as the sum of the effective molar volumes of the component ions at a given temperature. Based on this assumption, Jacquemin et al. have developed a group contribution method for the calculation of ionic liquid densities as a function of temperature at 0.1 MPa[25] or at higher pressures.[29] In this model, the effective molar volume of an n-alkylmethylimidazolium cation, $V_{[C_nmIm]^+}$, is calculated as the sum of the molar volumes of the methylimidazolium cation $[C_0mIm]^+$ and of the n $-CH_2-$ groups present in the alkyl chain:

$$V_{[C_nmIm]^+} = V_{[C_0mIm]^+} + nV_{-CH_2-} \quad (2)$$

Similarly, we consider the cation as the addition of several groups. For example, the molar volume of $[C_1COOC_2OC_2mIm]^+$, $V_{[C_1COOC_2OC_2mIm]^+}$, can be decomposed as:

Table 1. Structures, acronyms, and masses of the ionic liquids considered in this work.

Structure	Acronym	Mass [g mol^{-1}]
	$[C_1COOC_5mIm][C_8SO_4]$	419.56
	$[C_1COOC_5mIm][Tf_2N]$	490.42
	$[C_1COOC_2OC_2mIm][Tf_2N]$	492.39
	$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	564.50

$$V_{[C_1COOC_2OC_2mIm]^+} = V_{[C_0mIm]^+} + 5V_{-CH_2-} + V_{-O-} + V_{-COO-} \quad (3)$$

where V_{-O-} and V_{-COO-} are the contributions of the ether and ester groups, respectively.

According to Jacquemin et al.,[25] the molar volume of each group (V_{group}) can be expressed as a function of temperature:

$$V_{group}(T) = \sum_{i=0}^{2} C_i (T - 298)^i \quad (4)$$

where the parameters C_i are determined from experimental densities.

From the measured densities of $[C_1COOC_5mIm][Tf_2N]$ and $[C_1COOC_2OC_2OC_4mIm][Tf_2N]$, the coefficients C_i for the ether and ester groups were calculated. They are presented in Table 3 along with the parameters necessary for our prediction

Table 3. Parameters C_i associated with Equation (7), used to predict the effective molar volume of ions as a function of temperature at 0.1 MPa.

Group	C_0	$10^3 C_1$	$10^6 C_2$ [$cm^3 mol^{-1} K^{-1}$]	Ref.
$[Tf_2N]^-$	157.597	104.3	50.52	[29]
$[C_8SO_4]^-$	191.402	314.6	−914.0	[29]
$[C_0mIm]^+$	66.509	12.49	552.9	[29]
$-CH_2-$	16.967	13.99	−1.946	[29]
−COO−	21.680	31.01	−433.2	This work
−O−	4.571	1.869	20.58	This work

and already calculated in literature. From these data it was possible to predict the molar volumes of $[C_1COOC_2OC_2mIm][Tf_2N]$ and $[C_1COOC_5mIm][C_8SO_4]$ as a function of temperature. Average absolute deviations of 0.7% and 4.4% between the experimental molar volumes and the predicted values were obtained, respectively. The first value is coherent with the uncertainty of 0.5% claimed by Jacquemin et al.[25] The value of 4.4%, higher but acceptable, can be due to the contribution of the anion $[C_8SO_4]^-$, calculated by Jacquemin et al. using only few experimental data.[25]

Gas solubility

For each system studied, multiple experimental data points were obtained in the temperature interval between 303 K and 343 K in steps of approximately 10 K. The experimental solubilities for carbon dioxide, ethane, methane, and hydrogen in the different ionic liquids are reported in Tables 4, 5, 6, and 7. The results are given as Henry's law constants (K_H) and as mole fractions of solute (x_2). These last values are calculated from the experimental data of K_H (at slightly different total pressures) assuming a partial pressure of the gaseous solute equal to 0.1 MPa. The relative masses used are the ones recommended by IUPAC[30] and the values of the second virial coefficients for all the gases, necessary for the calculation of the compressibility factor and of the fugacity coefficient of the solute, were taken from the compilation by Dymond and Smith.[31]

Table 4. Experimental values of gas solubilities in $[C_1COOC_5mIm][C_8SO_4]$ expressed as both Henry's law constants K_H and as mole fraction x_2 corrected for a partial pressure of solute of 0.1 MPa. p is the experimental equilibrium pressure and the percent deviation is relative to the correlations of the data reported in Table 8.

Gas	T [K]	p [10^2 Pa]	K_H [10^5 Pa]	$10^{-3} x_2$	Deviation
CO_2	323.43	881.8	64.07	15.55	−2.4%
	323.45	702.9	61.31	16.25	+2.0%
	328.44	895.5	65.49	15.21	+0.7%
	328.44	714.2	65.67	15.17	+0.4%
	333.42	909.8	69.98	14.24	−0.1%
	333.45	725.5	69.94	14.25	+0.0%
	338.42	924.0	74.24	13.43	+0.5%
	338.46	737.0	75.89	13.13	−1.6%
	343.41	938.2	78.45	12.71	+2.0%
	343.45	748.2	81.09	12.29	−1.3%
C_2H_6	323.43	840.9	91.32	10.89	+0.2%
	323.43	908.0	92.00	10.81	−0.5%
	323.45	888.2	91.03	10.92	+0.6%
	328.42	922.6	97.93	10.16	−0.9%
	328.42	854.2	96.65	10.29	+0.4%
	328.45	902.4	96.67	10.29	+0.4%
	333.40	937.1	104.5	9.523	−1.7%
	333.40	867.6	102.4	9.718	+0.4%
	333.43	916.6	102.9	9.667	−0.1%
	338.41	951.1	106.0	9.392	+2.5%
	338.43	930.8	109.0	9.126	−0.4%
	343.40	965.7	114.2	8.719	+0.3%
	343.43	944.9	115.8	8.594	−1.1%
CH_4	323.44	796.2	360.3	2.775	+2.1%
	323.45	772.9	375.1	2.663	−2.0%
	328.43	808.4	371.8	2.689	+2.8%
	328.45	784.8	394.0	2.535	−2.9%
	333.43	820.7	389.7	2.566	+2.9%
	333.45	796.7	412.7	2.420	−2.8%
	338.44	833.0	414.1	2.415	+2.4%
	338.46	808.6	433.8	2.303	−2.2%
	343.43	845.3	446.9	2.238	+1.3%
	343.46	820.6	458.3	2.180	−1.4%

To correlate the solubility as a function of temperature, the Henry's law constants obtained from the experimental data were adjusted to a power series in $1/T$:

$$\ln(K_H/10^5) = \sum_{i=n}^{n} A_i (T)^{-1} \quad (5)$$

The coefficients A_i of Equation (5) as well as the average absolute deviations obtained are collected in Table 8. The average absolute deviations (average of the absolute value of the relative deviations on K_H) characterize the precision of the data, which is ca. 2% at most for the systems studied, except for the case of hydrogen in $[C_1COOC_5mIm][Ntf_2]$ for which a value of 5% was found. It was checked that this uncertainty is comparable to the statistical error estimation calculated by the propagation of the experimental uncertainty on pressure and temperature. There are strong motivations for studying the hydrogen solubility in ionic liquids, even if it is difficult to obtain very precise results. Hydrogenation reactions are of both fundamental and technological interest, and metal transition catalysis, classically used for these reactions, present major draw-

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Table 5. Experimental values of gas solubilities in $[C_1COOC_5mIm][Tf_2N]$ expressed as both Henry's law constants K_H and as mole fraction x_2 corrected for a partial pressure of solute of 0.1 MPa. p is the experimental equilibrium pressure and the percent deviation is relative to the correlations of the data reported in Table 8.

Gas	T [K]	p [10^2 Pa]	K_H [10^5 Pa]	$10^{-3}x_2$	Deviation
CO_2	303.43	585.9	34.20	29.10	−0.2%
	303.45	627.4	34.09	29.19	+0.2%
	313.44	609.1	40.38	24.66	+0.0%
	313.45	652.0	40.45	24.62	−0.1%
	323.42	631.8	47.39	21.02	−0.4%
	323.44	675.8	46.90	21.24	+0.7%
	333.45	654.2	55.03	18.11	−0.6%
	333.47	699.6	54.58	18.26	+0.2%
	343.44	676.0	62.92	15.84	−0.3%
	343.48	722.9	62.47	15.96	+0.5%
C_2H_6	303.36	819.0	86.28	11.59	−0.8%
	303.40	714.1	85.17	11.74	+0.5%
	303.41	714.3	85.70	11.67	−0.1%
	303.43	945.4	84.98	11.77	+0.8%
	313.10	739.0	96.84	10.33	−0.4%
	313.22	847.5	97.03	10.31	−0.5%
	313.44	978.4	96.83	10.33	+0.0%
	323.15	876.0	107.8	9.274	+0.7%
	323.20	764.5	108.8	9.196	−0.1%
	333.06	904.6	121.6	8.301	−0.1%
	333.08	789.1	120.5	8.221	+0.9%
	333.46	1043.9	122.7	8.151	−0.5%
	342.97	814.1	136.0	7.355	−0.4%
	343.50	1076.6	137.1	7.293	−0.7%
	343.02	932.8	134.2	7.452	+0.9%
CH_4	303.43	759.1	347.9	2.870	+1.8%
	303.44	720.6	354.1	2.820	+0.0%
	303.44	815.9	354.6	2.819	−0.2%
	313.44	842.8	392.9	2.545	−1.2%
	313.45	744.4	395.2	2.527	−1.8%
	323.42	768.0	431.7	2.313	+0.3%
	323.43	869.7	437.7	2.284	−1.1%
	333.42	896.5	484.7	2.115	+1.0%
	333.45	834.2	472.3	2.063	+3.7%
	343.44	923.5	567.2	1.764	−1.2%
	343.45	859.4	566.4	1.763	−1.1%
H_2	303.43	1327.9	1649.3	0.6063	+0.2%
	303.43	1221.5	1792.5	0.5579	−7.8%
	303.43	1013.9	1556.0	0.6427	+6.2%
	313.42	923.3	1659.7	0.6025	+4.9%
	313.43	1414.5	1833.9	0.5453	−5.1%
	313.44	1298.9	1658.6	0.6029	+4.9%
	323.42	1182.9	1681.6	0.5947	+8.6%
	323.43	983.6	1978.3	0.5055	−7.7%
	323.45	1501.1	1883.9	0.5308	−3.0%
	333.41	1371.2	1782.7	0.5609	+7.2%
	333.48	1044.2	2050.1	0.4878	−6.8%
	343.37	953.4	1987.4	0.5032	+0.3%

Table 6. Experimental values of gas solubilities in $[C_1COOC_2OC_2mIm][Tf_2N]$ expressed as both Henry's law constants K_H and as mole fraction x_2 corrected for a partial pressure of solute of 0.1 MPa. p is the experimental equilibrium pressure and the percent deviation is relative to the correlations of the data reported in Table 8.

Gas	T [K]	p [10^2 Pa]	K_H [10^5 Pa]	$10^{-3}x_2$	Deviation
CO_2	303.51	683.7	31.15	31.95	−0.3%
	303.53	753.0	31.00	32.11	+0.3%
	313.51	707.9	36.37	27.38	−0.2%
	313.54	779.3	36.25	27.47	+0.2%
	323.55	805.1	41.57	23.96	+0.3%
	323.55	731.8	41.70	23.89	+0.0%
	333.58	755.5	47.30	21.07	−0.3%
	333.63	831.0	47.17	21.13	+0.0%
	343.64	779.0	52.68	18.92	−0.2%
	343.68	856.6	52.45	19.01	+0.3%
C_2H_6	303.47	771.4	82.68	12.01	+0.6%
	303.47	482.1	83.66	11.87	−0.6%
	303.52	887.4	83.65	11.87	−0.5%
	313.45	797.0	90.26	11.01	+0.2%
	313.46	499.0	90.58	10.97	−0.2%
	313.53	916.9	89.39	11.12	+1.2%
	323.45	822.6	98.19	10.13	−0.8%
	323.48	515.7	97.07	10.24	+0.4%
	333.46	848.0	105.1	9.467	−0.8%
	333.46	532.3	103.1	9.647	+1.1%
	333.54	976.0	105.3	9.445	−1.0%
	343.48	549.0	110.0	9.044	+0.6%
	343.60	1005.5	111.0	8.969	−0.1%

Table 7. Experimental values of gas solubilities in $[C_1COOC_2OC_2OC_4mIm][Tf_2N]$ expressed as both Henry's law constants K_H and as mole fraction x_2 corrected for a partial pressure of solute of 0.1 MPa. p is the experimental equilibrium pressure and the percent deviation is relative to the correlations of the data reported in Table 8.

Gas	T [K]	p [10^2 Pa]	K_H [10^5 Pa]	$10^{-3}x_2$	Deviation
CO_2	303.45	542.2	27.37	36.53	−0.1%
	303.45	794.0	27.30	36.45	+0.1%
	313.46	561.4	32.11	31.15	−0.4%
	313.46	821.8	31.87	31.25	+0.4%
	323.47	580.5	37.57	26.62	−0.9%
	323.47	849.3	36.88	27.01	+1.0%
	333.47	599.5	43.80	22.83	−1.5%
	333.47	876.8	42.57	23.41	+1.3%
	343.48	904.0	48.42	20.59	+2.7%
	343.49	618.4	51.05	19.59	−2.6%
C_2H_6	303.45	543.7	58.05	17.11	+0.1%
	303.45	796.9	58.21	17.18	−0.2%
	313.45	824.3	65.19	15.34	−0.1%
	313.46	562.4	64.90	15.31	+0.4%
	323.45	851.6	73.06	13.69	−0.5%
	323.47	581.1	72.29	13.75	+0.6%
	333.46	878.9	81.33	12.30	−0.7%
	333.48	599.7	80.53	12.35	+0.4%
	343.46	905.9	89.09	11.22	+0.4%
	343.48	618.3	89.65	11.10	−0.2%

backs related with poor selectivity and recycling problems. Ionic liquids are now investigated as alternative solvents for catalytic hydrogenations,[32,33] and so knowledge on hydrogen solubility in these media is necessary. Morrissey et al.[34] have studied the selective hydrogenation of *trans*-cinnamaldehyde using two classical imidazolium-based ionic liquids ($[C_4mIm][Tf_2N]$ and $[C_4mIm][C_8SO_4]$) and the modified $[C_1COOC_5mIm][Tf_2N]$. In the three cases, 100% conversion was obtained. The selective formation of hydrocinnamaldehyde is of both academic and industrial interest. The higher selectivity for this component was obtained using $[C_1COOC_5mIm][Tf_2N]$ (94% selectivity instead of 87% in $[C_4mIm][Tf_2N]$ and 69% in $[C_4mIm]$-

Table 8. Parameters of Equation (8) used to smooth the raw experimental results from Tables 4–7 along with the percent average absolute deviation of the fit (AAD).

Ionic liquid	Gas	A_0	A_1	A_2	AAD
$[C_1COOC_5mIm][C_8SO_4]$	CO_2	24.53	-1.21×10^4	1.79×10^6	1.1%
	C_2H_6	8.012	-1.01×10^3	-4.00×10^4	0.6%
	CH_4	31.59	-1.59×10^4	2.46×10^6	2.3%
$[C_1COOC_5mIm][Tf_2N]$	CO_2	8.322	-1.31×10^3	-4.46×10^4	0.3%
	C_2H_6	9.769	-2.09×10^3	1.45×10^5	0.5%
	CH_4	20.93	-8.39×10^3	1.16×10^6	1.3%
	H_2	9.024	-4.90×10^2	–	5.2%
$[C_1COOC_2OC_2mIm][Tf_2N]$	CO_2	4.048	1.15×10^3	-4.07×10^5	0.2%
	C_2H_6	5.227	3.22×10^2	-1.72×10^5	0.6%
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	CO_2	10.65	-2.99×10^3	2.30×10^5	1.1%
	C_2H_6	9.091	-1.98×10^3	1.38×10^5	0.3%

$[C_8SO_4]$). According to Morrissey et al.,[34] this could be due to a variation of the hydrogen solubility. This is not confirmed by the present study, during which comparable solubilities were measured for hydrogen in the three ionic liquids: at 303 K, a H_2 mole fraction of 6×10^{-4} in $[C_1COOC_5mIm][Tf_2N]$ and of 7×10^{-4} in $[C_4mIm][Tf_2N]$[35] and at 313 K, a H_2 mole fraction of 5.5×10^{-4} in $[C_1COOC_5mIm][Tf_2N]$ and of 5.3×10^{-4} in $[C_4mIm][C_8SO_4]$.[23]

The experimental solubilities of carbon dioxide and ethane in the four ionic liquids are presented in Figure 1. In all of the considered liquids, carbon dioxide is more soluble (mole fraction between 1.6 and 4×10^{-2}) than ethane (mole fraction between 0.7 and 1.7×10^{-2}), which is more soluble than methane (mole fraction between 1.7 and 2.9×10^{-3}). As for other ionic liquids based on the 1-alkyl-3-methylimidazolium cation, hydrogen is the least soluble gas.[24–27,36–38] Thus, the chemical modification of the alkyl chain does not seem to cause major modification of the solvation properties of ionic liquids. For all the tested systems, the mole fraction solubility decreased with temperature. The solvation takes place preferentially at lower temperatures, which corresponds to an exothermic process.

As can be observed in Figure 1, the octylsulfate anion decreases the solubility of carbon dioxide (nearly 25%) while methane and ethane are more soluble in $[C_1COOC_5mIm][C_8SO_4]$. The decrease of the carbon dioxide solubility in octylsulfate-based ionic liquids has also been reported in the literature:[23] this gas is four times more soluble in $[C_4mIm][Tf_2N]$ than in $[C_4mIm][OcSO_4]$. The inclusion of ether groups and the increase of the alkyl chain length in the imidazolium ring in the three Tf_2N-based ionic liquids led to an increase of carbon dioxide and ethane solubility. Similar results have been found for ionic liquids with a Tf_2N^- anion but without an ether or ester moiety: the carbon dioxide, ethane, and butane solubility increased with the alkyl chain length,[39] at least in alkyl chains of up to eight carbon atoms.

In Figure 2, the present data are compared with gas solubilities in alkylimidazolium-based ionic liquids reported in literature. The solubilities measured in the ester- and ether-substituted ionic liquids are of the same order of magnitude as those in ionic liquids based on the alkylimidazolium cations and having the same number of carbon in the alkyl chain. Particularly, in the studied temperature range, the carbon dioxide solubility in $[C_1COOC_5mIm][Tf_2N]$ (seven carbons in alkyl chain) is between that observed in $[C_6mIm][Tf_2N]$ and in $[C_8mIm][Tf_2N]$. The ethane solubility in $[C_1COOC_5mIm][Tf_2N]$ is slightly lower (12% at 325 K) than that measured for $[C_6mIm][Tf_2N]$.[37]

The variation of the solubility with temperature, expressed as Henry's law constant, is directly related to the thermodynamic properties of solvation, which in the present case (gaseous solutes at low pressures) are practically identical to the thermodynamic properties of solution.[40] The Gibbs energy of solvation is given by:

$$\Delta_{sol}G^{\infty} = RT\ln(K_H/p^0) \quad (6)$$

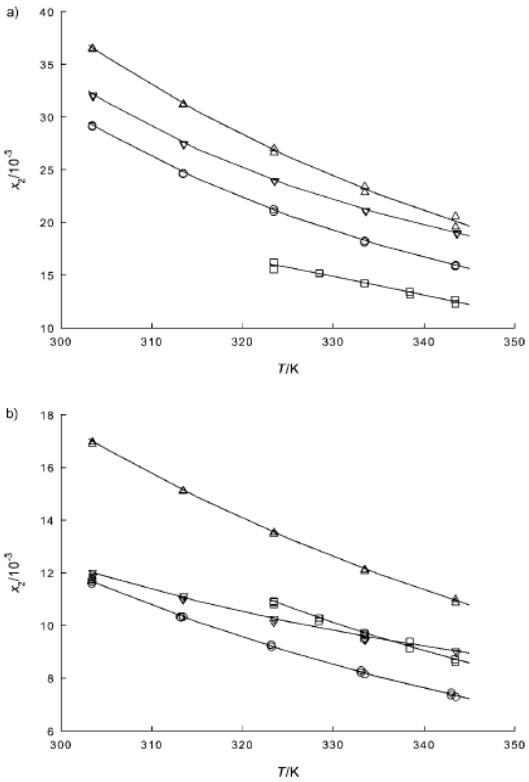

Figure 1. Mole fraction solubilities of a) CO_2 and b) C_2H_6 at 0.1 MPa partial pressure of the solutes as a function of temperature. □: $[C_1COOC_5mIm][C_8SO_4]$; ○: $[C_1COOC_5mIm][Tf_2N]$; ▽: $[C_1COOC_2OC_2mIm][Tf_2N]$; △: $[C_1COOC_2OC_2OC_4mIm][Tf_2N]$. Lines represent smoothed data; parameters in Table 7.

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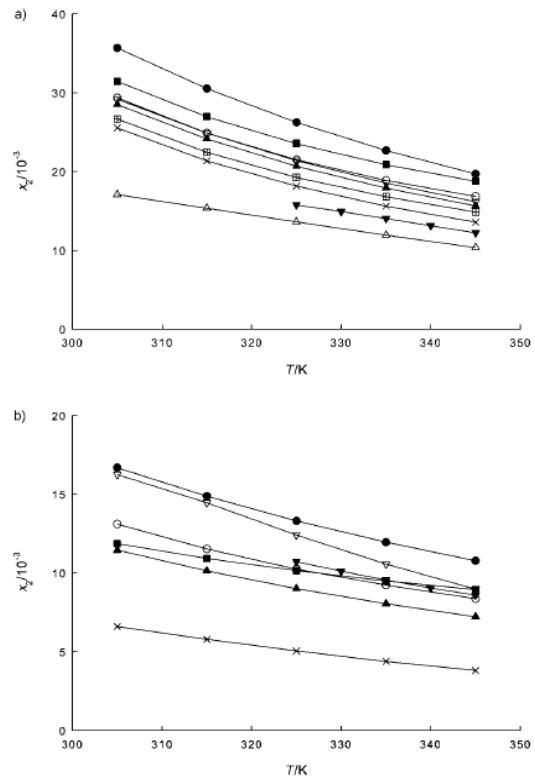

Figure 2. Mole fraction solubilities of a) CO_2 and b) C_2H_6 in various ionic liquids as a function of temperature: ▲: $[C_1COOC_5mIm][Tf_2N]$; ▼: $[C_1COOC_5mIm][C_8SO_4]$; ■: $[C_1COOC_2OC_2mIm][Tf_2N]$; ●: $[C_1COOC_2OC_2OC_4mIm][Tf_2N]$; ×: $[C_1C_2Im][Tf_2N]$;[44] □: $[C_4mIm][Tf_2N]$;[26] ○: $[C_6mIm][Tf_2N]$;[45] ▽: $[C_8mIm][Tf_2N]$;[46] △: $[C_4mIm][C_8SO_4]$.[27]

where p^0 is the standard state pressure. The partial molar differences in enthalpy and entropy can be obtained by calculating the corresponding partial derivatives of the Gibbs energy with respect to temperature:

$$\Delta_{sol}H^{\infty} = -T^2\partial/\partial T(\Delta_{sol}G^{\infty}/T) = -RT^2\partial/\partial T[\ln(K_H/p^0)] \quad (7)$$

$$\Delta_{sol}S^{\infty} = (\Delta_{sol}H^{\infty} - \Delta_{sol}G^{\infty})/T = -RT\partial/\partial T[\ln(K_H/p^0)] - R\ln(K_H/p^0) \quad (8)$$

The values of the Gibbs energy, enthalpy, and entropy of solvation are given in Table 9 for the considered systems at several discrete temperatures.

For all of the tested systems, in the temperature range considered, the enthalpy of solvation is negative, corresponding to an exothermic process, and varies between −5 and −15 kJ mol^{-1}. All of the entropies are the same order of magnitude, between −55 and −93 J K^{-1} mol^{-1}. The thermodynamic properties of solvation provide valuable information about both the solute–solvent interactions and about the molecular structure of the solutions: the enthalpy of solution is closely related with the crossed gas–ionic liquid molecular interactions and the entropy of solvation gives indications about the structure of the solvent molecules surrounding the solute.[41] A more detailed analysis of the data presented in Table 9 indicates that the evolution of these quantities with temperature is function of the system. In particular, for carbon dioxide, a behavior different from other gases is observed, both for the enthalpy and for the entropy for the octylsulfate-based and Tf_2N-based ionic liquids. These two thermodynamic properties are constant (or slightly increase) with temperature in the Tf_2N-based ionic liquids while they decrease with temperature in the case of octylsulfate ionic liquid. These differences, due to the change of the anion, are not observed for the two other gases for which enthalpy and entropy are constant or slightly decrease with temperature for the four studied ionic liquids. These observations probably mean that the solvent–solute interactions and structure of the solutions are of different nature in the three gases and that, in the case of carbon dioxide, they are a function of the anion of the ionic liquid.

The derived thermodynamic properties calculated for the functionalized ionic liquids can be compared to those of alkylimidazolium ionic liquids (Figure 3). Similar orders of magnitude are found for the ionic liquids studied here and for nonfunctionalized imidazolium-based ionic liquids, corresponding to comparable carbon dioxide solubilities. The entropic term ($T\Delta S$) is always higher in absolute value, typically by a factor of two, than the enthalpy (ΔH). The presence of the $[C_8SO_4]^-$ anion leads to less-negative enthalpies and entropic terms. It is the less favorable enthalpy that explains the decrease of carbon dioxide solubility observed with this anion. The higher carbon dioxide solubility observed for the ionic liquids with ester and ether groups is due to a larger negative enthalpic term, which corresponds to more favorable solute–solvent interactions. It is observed in alkylimidazolium ionic liquids that the solubility of carbon dioxide increases with the length of the alkyl side chain but only up to 6 or 8 carbon atoms, and then it remains almost constant.[42] Herein, it is observed that the carbon dioxide solubility continues to increase when the functionalized alkyl side chain increases and this larger solubility corresponds to a more-negative enthalpy of solvation. This fact is probably explained by a specific interaction between carbon dioxide and the ester and/or ether groups present in the ionic liquids studied here that will contribute favorably to the solubility of this gas.

Conclusions

The solubilities of carbon dioxide, methane, and ethane are of the same order of magnitude in the four ionic liquids, including the biodegradable example $[C_1COOC_5mIm][C_8SO_4]$, as in conventional alkylimidazolium ionic liquids. The chemical modifications of the alkyl side chain which increase biodegradation in ionic liquids (i.e., ester and ether inclusion) did not introduce a significant change in the solvation properties of the ionic liquid. Carbon dioxide is the most soluble gas, followed by ethane and methane; hydrogen being the least soluble gas. The variation with temperature of the solubility allowed a study of the thermodynamic properties of solvation. For carbon dioxide, a different behavior is observed, both for

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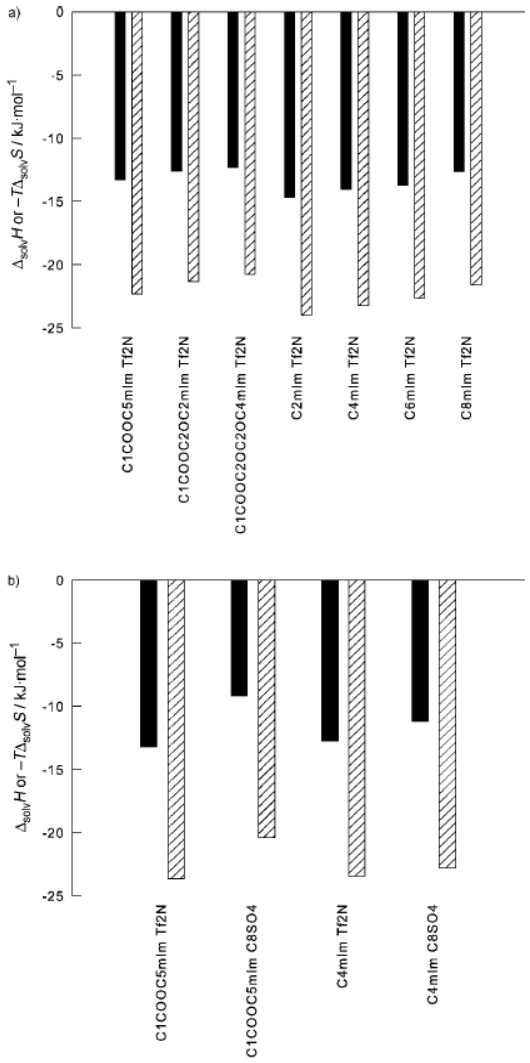

Figure 3. Comparison of the thermodynamic properties of solvation for carbon dioxide in different ionic liquids at a) 305 K and b) 325 K. Black bars: $\Delta_{solv}H$; striped bars: $-T\Delta_{solv}S$. Data from the literature are taken from References [23–27,45].

viscosity of the sample was not considered, as it affects the density by less than 0.01%.

Gas solubility

The experimental method used for the present gas solubility measurements was based on an isochoric saturation technique that has been described in previous publications.[45,46,35] In this technique, a known amount of gaseous solute is put in contact with a precisely determined quantity of degassed solvent at a constant temperature inside an accurately known volume. When thermodynamic equilibrium is attained at a given temperature, the pressure above the liquid solution is constant and is directly related to the solubility of the gas in the liquid. A large surface of contact and an appropriate stirring of the liquid phase guarantee a sufficient exchange between the liquid solution and the gaseous phase in equilibrium with it.

The quantity of ionic liquid introduced in the equilibration cell, V_{liq}, was determined gravimetrically. The amount of solute present in the liquid solution, n_2^{liq} (subscript 2 stands for solute and subscript 1 for solvent), was calculated by the difference between two pVT measurements: the first measurement was taken when the gas was introduced into a calibrated bulb with volume V_{GB} and the second after thermodynamic equilibrium was reached:

$$n_2^{liq} = \frac{p_{ini}V_{GB}}{[Z_2(p_{ini},T_{ini})RT_{ini}]} - \frac{p_{eq}(V_{tot}-V_{liq})}{[Z_2(p_{eq},T_{eq})RT_{eq}]} \quad (9)$$

where p_{ini} and T_{ini} are the pressure and temperature in the first pVT determination and p_{eq} and T_{eq} the pressure and temperature at the equilibrium. V_{tot} is the total volume of the equilibration cell and Z_2 is the compressibility factor for the pure gas. The solubility could then be expressed as mole fraction:

$$x_2 = \frac{n_2^{liq}}{(n_1^{liq}+n_2^{liq})} \quad (10)$$

or as Henry's law constant:

$$K_H \equiv \lim_{x_2\to 0}\frac{f_2(p,T,x_2)}{x_2} \simeq \frac{\phi_2(p_{eq},T_{eq})p_{eq}}{x_2} \quad (11)$$

where f_2 is the fugacity of the solute and ϕ_2 its fugacity coefficient. The fugacity coefficient, which was very close to unity in the present cases, was included in the calculation of the Henry's law constants for all the gases.

Acknowledgement

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Keywords: biodegradation · gases · ionic liquids · solvent effects · thermodynamics

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Octanol-water partition coefficients of biodegradable ionic liquids

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Keywords: ionic liquid, toxicity, biodegradability, octanol-water partition coefficient

Abstract

Ionic liquids have attracted great attention as green solvents mainly because of their non-volatility, thermal stability and non-flammability. Even though, their impact on organisms and environment is still rarely studied. The octanol-water partition coefficient (K_{OW}) is a key parameter to assess these impacts. It is an operational measuring physico-chemical property that describes the hydrophobicity/hydrophilicity of a compound and is correlated with biological activity, soil sorption and parameters predicting biological, biochemical and toxic effects [1]. Low K_{OW} values are associated to a limited rate of passive diffusion across a biological membrane and low risk to accumulate in the environment [2].

Gathergood *et al.* [3] have introduced in the alkyl side chains of methylimidazolium-based ionic liquids, ester and/or ether groups, which can be recognized by enzymes, and have successfully synthesized a new series of ionic liquids whose biodegradability is greatly improved.

In this study, we have used the slow-stirring method [4] to measure K_{OW} for three these modified ionic liquids, with bis(trifluoromethylsulfonyl)imide anion. The K_{OW} values vary slightly with the concentration of ionic liquid (around 10^{-3} mol.L^{-1}) and are low, typically ranging from 0.05 to 1.82 depending on the ester side chains on the imidazolium cation. The same order of magnitude for the partition coefficient was obtained for non-functionalised ionic liquids [5]. This means that the inclusion of ester and/or ether groups in the alkyl chain of ionic liquids does not significantly change their impact on the organisms and on the environment, as indicated by the octanol-water partition coefficient. Ionic liquids have lower K_{OW} than many traditional halogen solvents and solvents of high molecular weight, meaning that these components are much “greener” from this aspect.

1. Introduction

In recent years, ionic liquids (ILs) have been investigated as “green solvents” mainly

because of their non-volatility, thermal stability and nonflammability. Their negligible vapour pressure minimizes their impact on human being. However a “real green solvent” should also be non-persistent in environment. According to recent studies [6,7,8] classical alkylimidazolium-based ILs are poorly biodegradable. Gathergood *et al.* [3] have synthesized a new series of ILs, by introducing ester / ester and ether group in the alkyl side chains of methylimidazolium-based ILs. Because enzymes can recognize ester and ether group, the biodegradability of the new ILs is greatly improved.

The octanol-water partition coefficient is an important factor determining movements and distributions of chemicals in the environment [9]. It is also an important characteristic from biochemical point of view [10]. It is accepted for a long time as one of the quantitative physical properties that correlates best with biological activity, the soil sorption constant, water solubility and parameters predicting biological, biochemical and toxic effects [1,5]. It is widely used as a parameter for quantitative structure–activity relationship (QSAR) and plays an important role in the early stages of an environmental risk assessment for a chemical. [1,11] Octanol-water partition coefficient is defined as the ratio of the equilibrium concentrations of the test substance in 1-octanol saturated with water (C_O) and in water saturated with 1-octanol (C_W) [4].

$$K_{OW} = C_O / C_W \qquad (1)$$

It should be constant for any solute at equilibrium distribution in the solution sufficiently dilute at a certain temperature (Nernst distribution law) [1,5,9].

A large number of methods for determining K_{OW} are proposed in literature [1,9], which can be classified in two categorises – measurement and predictive methods. The measurement methods include direct and indirect experimental methods. The predictive methods make use of varies of structural information to calculate $K_{OW.}$ The most used measurement methods (shake-flask, slow-stirring and high-performance liquid chromatography) are proposed as standard procedures in *Organization for Economic Cooperation and Development (OECD) guidelines for testing chemicals* [4].

Despite ILs are considered as “green solvent”, their impacts on the organism and environment are just beginning to be studied. Even K_{OW} is a key parameter of assessment these impacts, it has been investigated rarely for ILs. Ropel *et al.* [5], Lee and Lee [12] and Choua *et al.* [13] have used direct measurement methods to test imidazolium ILs. Domanska *et al*. [14], and Chapeaux *et al.* [15] have implied indirect methods to obtain the K_{OW} of some classic imidazolium, pyridinium and quaternary ammonium-based ILs, from measuring the solubility of ILs in water and in 1-octanol. Stepnowski and Storoniak [16] have estimated K_{OW} of some imidazolium ILs from their structures. The K_{OW} values of methylimidazolium-based ILs from literature are presented in Table 3. In spite the discrepancy is huge, some tendencies can be observed. Except $[C_6mIm][Ntf_2]$ and $[C_8mIm][Ntf_2]$, for most of imidazolium and quaternary ammonium ILs, K_{OW} is lower than 1. Pyridinium ILs have larger K_{OW} than imidazolium ILs. For the ILs with same anion, K_{OW} increases as the alkyl side chain on the cation increases. K_{OW} of ILs with $[Ntf_2^-]$ are larger than those of ILs with other anions, eg. $[Br^-]$, $[BF_4^-]$, $[NO_3^-]$, $[Cl^-]$ and $[PF_6^-]$, while those of ILs with $[Br^-]$, $[NO_3^-]$ or $[Cl^-]$ are extremely low.

In this study, we have used the slow-stirring method to measure the K_{OW} of three modified methylimidazolium-based ILs (ester and/or ether groups introduced in the alkyl side chains of cation), with the bis(trifluoromethylsulfonyl)imide ($[Ntf_2^-]$) anion. The experimental technique is tested by measuring K_{OW} of imidazole. The effect of the different ester side

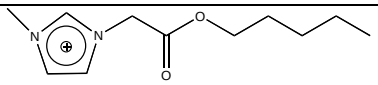
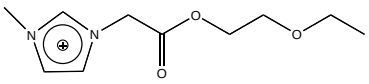
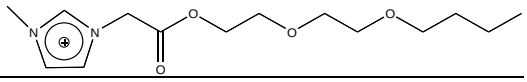
chains on the imidazolium rings of the cations on K_{OW} is studied.

2.Experimental

2.1 Materials

The ILs used in this study are provided by Dublin City University, Ireland. They were synthesised from alcohols and glycols, thus leading to imidazolium ILs with oxygen in the side chain of the cation. [17,18] They were dried at 85 °C for several days before use. The anion is bis(trifluoromethylsulfonyl)imide ([Ntf_2^-]) and the cations are listed in Table 1. 1-octanol (CAS N° 111-87-5, Fluka, grade GC, purity>99.5%) and imidazole (CAS N° 288-32-4, Aldrich, purity 99%) were used without further purification.

Table 1. Names and structures of the cations of the tested ILs

Abbreviation	Name of cation	Structure of cation
[C_5-C_2O_2-mIm]	3-methyl-1-(pentoxycarbonylmethyl) imidazolium	
[C_2-C_4O_3-mIm]	3-methyl-1-(ethoxyethoxycarbonyl methyl) imidazolium	
[C_4-C_6O_4-mIm]	3-methyl-1-(2-(2-butoxyethoxy) ethoxycarbonylmethyl)imidazolium	

2.2 Experimental technique

The test follows the *OECD Guideline 123* and is made at room temperature (22 ± 1 °C). Both water and 1-octanol are mutually saturated prior the experiment. Two stock bottles, one containing 1-octanol and a sufficient quantity of water, and the other containing water and a sufficient quantity of 1-octanol, are stirred using a Teflon coated stir bar for at least two days, and then stand long enough to allow the phases to separate.

Depending on the detection limit and solubility of the IL in octanol, different low concentration solutions of the IL were prepared using 1-octanol pre-saturated with water as solvent. 5 ml water pre-saturated with 1-octanol was placed in a 7 ml glass vial with an open-top screw cap sealed with septum. 1.5 ml of the solution of IL in 1-octanol saturated with water was carefully added against the wall of vial, close to the water surface, reducing the possibility of formation of microdroplets from 1-octanol in the water phase. The vial was nearly filled for minimizing the vapour phase. It was closed tightly and stirred slowly using a Teflon coated stir bar for at least 48 hours, avoiding the vortex depth exceeding 1 cm. The two phases were allowed to separate for at least 2 hours. Each concentration was performed with 3 independent slow-stirring replicates and the standard deviation between the different replicates (*SD*) was calculated. 1-octanol rich phases were withdrawn with all glass-metal syringes.

Concentrations of ILs in each phase were measured using UV-vis spectroscopy (*UVIKON 941*). Sample was diluted if its concentration exceeded the calibration range.

3. Results and discussion

We have used imidazole to test our protocol. By comparing the experimental K_{OW} value (0.90±0.09) with literature data (0.83) [9], we can conclude that our experimental technique is adequate.

Experimental K_{OW} values of the tested ILs increase with the concentration, despite it is as low as of 10^{-3} mol.L^{-1}. This variation was also observed in literature using direct measurement methods [5,12,13]. The authors have attributed it to the dissociation of ILs in water-rich phase. Accordingly we report the data as a function of the equilibrium concentration of IL in the water rich phase (C_W). The experimental values of K_{OW} and the standard deviations are listed in Table 2 and plotted in Fig. 1.

K_{OW} values range from 0.05 to 1.82, depending on the different ester side chains in the imidazolium rings of the cations. [C_2-C_4O_3-mIm][Ntf_2] have the lowest K_{OW} value, when [C_5-Ester-mIm][Ntf_2] have the largest K_{OW} value. The values increase with the length of apolar part - alkyl chain, beside the ether/ester function, not with the total length of the side chain of the imidazolium ring.

K_{OW} of some classic 3-methyl-1-alkyl-imidazolium ([C_nmIm]) ILs are listed in Table 3 and Figure 1. [C_2-C_4O_3-mIm][Ntf_2] and [C_2mIm][Ntf_2], [C_4-C_6O_4-mIm][Ntf_2] and [C_4mIm][Ntf_2], [C_5-C_2O_2-mIm][Ntf_2] and [C_6mIm][Ntf_2] present very similar K_{OW} values. We can conclude that the introduction of ester and/ether in the alkyl chain does not gravely change this character.
This result corresponds to the conclusion of Soskic and Plavsic [19]: the contributions to K_{OW} of heteroatom may be negative. In the case of these three ILs, the contributions of total (C_nO_m) groups beside the apolar part in the side chain, are the addition of carbon atoms and oxygen atoms, hence finally negligible.

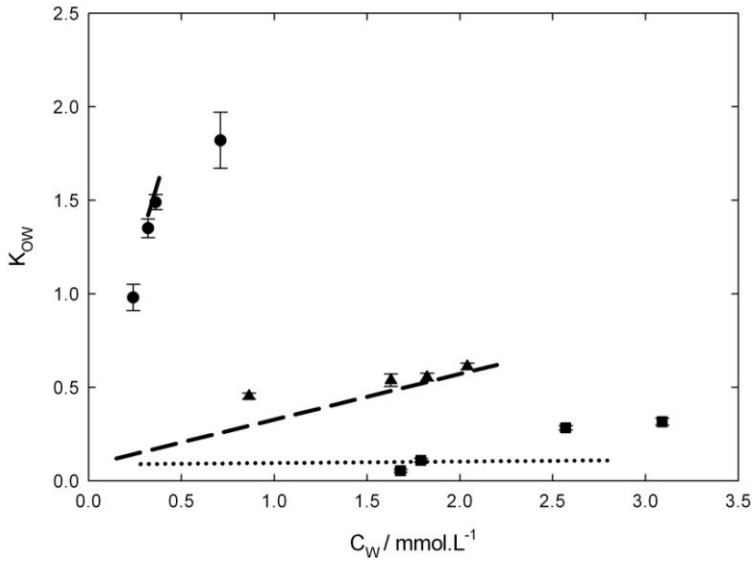


Figure 1. K_{OW} values at 22± 1 °C of ILs as a function of concentration of ILs in water-rich phase (C_W): ●, [C_5-C_2O_2-mIm][Ntf_2]; ■, [C_2-C_4O_3-mIm][Ntf_2]; ▲, [C_4-C_6O_4-mIm][Ntf_2]. Lines represent literature values [5]: dotted line, [C_2mIm][Ntf_2]; long dash line, [C_4mIm][Ntf_2]; solid line, [C_6mIm][Ntf_2].

Table 2. Experimental values of K_{OW} and the *SD* between different replicates

IL	C_W/mmol.L^{-1}	K_{OW}	*SD*	C_W/mmol.L^{-1}	K_{OW}	*SD*
[C_5-C_2O_2-mIm][Ntf_2]	0.24	0.98	0.07	0.36	1.49	0.04
	0.32	1.35	0.05	0.71	1.82	0.15
[C_2-C_4O_3-mIm][Ntf_2]	1.68	0.05	0.01	2.57	0.28	0.01
	1.79	0.11	0.01	3.09	0.32	0.02
[C_4-C_6O_4-mIm][Ntf_2]	1.82	0.55	0.02	0.86	0.45	0.02
	2.04	0.61	0.02	1.63	0.54	0.03

Table 3. K_{OW} of [C_nmIm] ILs between 20°C – 30 °C in literature

IL	K_{OW}	IL	K_{OW}
[C_2mIm][Ntf_2]	0.09-0.11[4][a]	[C_3mIm][BF_4]	0.0182[15][e]
[C_4mIm][Ntf_2]	0.11-0.62[4] 8.976[11][b]	[C_4mIm][BF_4]	0.0363[15]; 0.0030[4]
[C_6mIm][Ntf_2]	1.42-1.66[4]	[C_5mIm][BF_4]	0.0813[15]
[C_8mIm][Ntf_2]	6.3-11.1[4]	[C_6mIm][BF_4]	0.1950[15]
[C_4mIm][Cl]	0.0040[4] 0.48[13]	[C_2mIm][PF_6]	0.012-0.015 [12][c]
[C_8mIm][Cl]	0.54[13][d]	[C_4mIm][PF_6]	0.005-0.02 [12] 0.0220[4] 0.093[11]
[C_{10}mIm][Cl]	0.52[13]	[C_4mIm][NO_3]	0.0038[4]
[C_{12}mIm][Cl]	0.73[13]	[C_4mIm][Br]	0.0033[4]

[a] slow-stirring method ; [b c] shake-flask method ; [d e] predictive method

Table 4. K_{OW} values of some organic solvents [9]

Compound	K_{OW}	Compound	K_{OW}
ethanol	0.57	1,1-dichloroethylene	123
acetone	0.50	trichloroethylene	263
1-butanol	6.92	hexachlorobenzene	275423

Compared to some classical solvents (Table 4), K_{OW} values of ILs are similar to those of ‘small’ polar solvents (except halogen solvents), much lower than those of ‘big’ polar solvents and apolar solvents. From the aspect of K_{OW}, ILs is much “greener” than many traditional solvents.

In a word, the values of K_{OW} for all the methylimidazolium-based ILs are lower than 10. It is clear that they are not lipophilic. We can predict that in a general way, these ILs have low potential to accumulate or concentrate in the environment and organism. They have low rate of passive diffusion across a biological membrane, low possibility of being stored in fat depots or in other lipophilic sites such as membranes or lipoproteins. If the ILs are absorbed in vertebrates, they will tend to dissolve more (or nearly same) in blood water and will associate less with lipoproteins and membranes of blood cells, but difficult to cross the blood-brain barrier to move into the brain. For aquatic organisms, low biocentration factor (BCF) are associated with low K_{OW} values. If these ILs enter terrestrial ecosystems, they tend to dissolve in water and are adsorbed to soil colloids only to a limited degree. As consequence, they have low possibility of persistently transferring along terrestrial food chains.

4. Conclusion

The measurement of K_{OW} is difficult and the result can be affected by many uncerterties, so the experimental data from different authors present huge discrepancies. However some tendencies can be observed. The values of K_{OW} for all the methylimidazolium-based ILs tested and calculated are lower than 10, which means that they are not lipophilic. The [Ntf_2^-] based ILs are more lipophilic than those with other anions. For the ILs with ester and/or ether group in the alkyl side chain of cation, the values of K_{OW} increase with the length of alkyl chain beside the ester/ether group, not with the total length of side chain at the imidazolium ring of cation. The contribution of oxygen atom is negative and that of the total introduced groups beside the apolar part is negligible. All of these ILs have low environmental risk.

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Influence of Oxygen Functionalities on the Environmental Impact of Imidazolium-based Ionic Liquids

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ABSTRACT

Several physico-chemical properties relevant to determine the environmental impact of ionic liquids – aqueous solubility, octanol-water partition coefficient and diffusion coefficients in water at infinite dilution – together with toxicity and biodegradability of ionic liquids based on 1-alkyl-3-methylimidazolium cations with or without different oxygenated functional groups (hydroxyl, ester and ether) are studied in this work. The presence of oxygen groups on the imidazolium cation reduces the toxicity of ionic liquids 1-alkyl-3-methylimidazolium with bis(trifluoromethylsulfonyl)imide or octylsulfate anions and simultaneously decreases the value of their octanol-water partition coefficient. The presence of ester functions renders the ionic liquids more easily biodegradable, especially for long alkyl side-chains in the cation but leads to hydrolysis with the formation of reaction products that accumulate. The imidazolium ring is resistant to biodegradability and to abiotic degradation. The oxygen functionalised ionic liquids are more soluble in water and, diffuse more slowly in this medium.

Keywords: ionic liquids, physico-chemical properties, environmental impact, aqueous solubility, octanol-water partition coefficient, diffusion coefficients in water

1. Introduction

Ionic liquids can be used in a variety of applications in domains like catalysis, synthesis, separation or extraction because of their unique properties [1]. Their negligible vapour pressure makes them attractive to replace traditional volatile organic compounds but their wide use at an industrial scale requires further information on their environmental fate, toxicity and impact on ecosystems and human health. Although their physico-chemical properties minimize their diffusion into the atmosphere, their presence in the environment may become a reality, for example in the case of accidental release or at the end of their life cycle.

The few published studies on the environment impact of ILs have enhanced the poor biodegradability of those based on the 1-alkyl-3-methylimidazolium cation [2], the most commonly used at present. Toxicological studies have also shown that imidazolium-based ILs with alkyl chains longer than C6, for example 1-decyl-3-methylimidazolium tetrafluoroborate, $[C_{10}mIm][BF_4]$, and 1-methyl-3-tetradecylimidazolium chloride, $[C_{14}mIm][Cl]$, present high levels of toxicity to microorganisms, avoiding their biodegradation in the environment [3,4]. The experimental study of Deng et al. [5] showed that for the case of imidazolium-based ILs, the introduction of ester groups does not significantly affect physico-chemical properties as the density or the solubility of gases like carbon dioxide. Furthermore, Pensado et al. [6] used molecular simulation and showed that the molecular properties of the functionalised ionic liquids do not differ significantly from those of the non-functionalised ones although the molecular structure is slightly different with altered relative sizes of the polar and nonpolar domains in the ILs [7].

The development of new ILs with low environmental impact (easily biodegradable, non-toxic, with low bioaccumulation levels) seems to be one of the requirements for their more frequently use. This implies a rational molecular design with the aim of combining environmental scale requirements and human health security without loosing the chemical performance. Several strategies have been developed to increase the ILs biodegradability by modifying the chemical structures of the cation (imidazolium, pyridinium, ammonium families) and/or anion. For example, Gathergood and Scammells [8,9] have shown that functionalization (addition of ester or ether groups) of the alkyl side chains of

1-alkyl-3-methylimidazolium-based ILs can greatly improve their biodegradability. The nature of the anion appears also as fundamental: the first 'ready biodegradable' ionic liquid was prepared using n-octyl sulfate as anion [10].

In most of the studies published, the criteria to assess biodegradability are based on the determination of parameters like the dissolved organic carbon (DOC), the carbon dioxide production or the oxygen uptake by a mixed microbial population issued from a wastewater treatment plant as recommended by several OECD guidelines [11]. These parameters are followed at regular intervals during a pre-determined period of time and define the biodegradation of the ILs. For example, they are considered as 'readily biodegradable' if the biodegradation level is higher than 60% within 28 days. Except in the case of complete mineralization, metabolism can lead to the formation of transformation products more toxic, more persistent and/or more hazardous than the starting material, a fact that is not only taken into account in the tests recommended by OECD. This is one of the reasons why detailed investigations on biodegradation pathways of ILs are crucial. The identification of the chemical structures of the transformation products formed as well as the monitoring of IL disappearance kinetics are necessary for a better understanding of the potential environmental behavior of ILs and their global impact. The toxicity of these compounds, if identified, can therefore be also tested. This approach has been investigated in a few other studies using mainly activated sludge microbial community and NMR or GC-MS as analytical tools [2,12-16]. Under these conditions, the difficulty is to know which microorganism is responsible of the degradation process, in particular with the objective of developing biodepollution treatments.

In addition to the toxicology and biodegradability of ILs, there are key physico-chemical properties that allow the characterisation of the transfer and transport of these chemicals in the environment. These properties include the vapour pressure, aqueous solubility, octanol-water partition coefficient and diffusivity in water [17,18]. The octanol-water partition coefficient (K_{OW}) is defined as the ratio of the equilibrium concentrations of the test substance in 1-octanol saturated with water (C_O) and in water saturated with 1-octanol (C_W) [19]. This parameter is directly related with the possibility of a chemical to accumulate in organisms because water-saturated octanol is considered as a realistic model

of the physico-chemical environment in living organisms [20]. Diffusion of chemicals in water is a very important physical property that allows to understand their transport in environmental compartments, both water and soil. These physico-chemical properties are seldom determined for ionic liquids, the data being often imprecise and scattered, especially in the case of octanol-water partition coefficients [21]. The diffusion coefficient of ILs in water is rarely reported, the only ILs studied being based on 1-alkyl-3-methylimidazolium cations [22-24].

The objective of the present work is to study the environmental impact of a carefully chosen selection of six 1-alkyl-3-methylimidazolium-based ILs with alkyl side chains of various lengths, modulated or not by the insertion of oxygenated functional groups in order to increase the chemical reactivity and the biodegradability (esters and/or ether moieties). We have focussed on the bis(trifluoromethylsulfonyl)imide anion ($[Tf_2N]$) but the octylsulfate anion ($[C_8SO_4]$) was also studied for comparison. One of the imidazolium ILs based on this octylsulfate anion previously described as "readily biodegradable" was included in this work [10]. Different data describing the environmental impact of these ILs are measured: aqueous solubility, octanol-water partition coefficient, diffusion coefficient in water, biodegradation pathways with different pure microbial strains and microbial toxicity. The originality of the study is the simultaneous use of biological and physico-chemical tools to characterize the environmental impact of ILs.

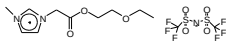
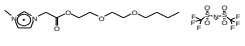
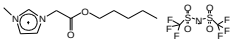
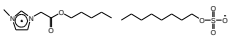
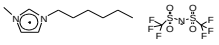
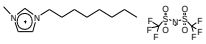
2. Experimental Section

2.1. Materials

The structures and abbreviations of the ILs used in this study are listed in Table 1. The functionalized ILs and 3-methyl-1-(carboxymethyl)imidazolium chloride ($[C_1COOHmIm][Cl]$) were provided by N. Gathergood's team, Dublin City University, Ireland. The functionalised ILs were synthesised from alcohols and glycols, thus leading to imidazolium ILs with oxygen in the side chain of the cation [25]. $[C_6mIm][Tf_2N]$ and $[C_8mIm][Tf_2N]$ were prepared from the appropriate chloride salt using the procedure

described by Bonhôte et al. [26]. Their purity is 99% as determined by ^{1}H NMR. [C_1COOHmIm][Cl] was also synthesised at Dublin City University from 1-methylimidazole with 2-chloroacetic acid and its purity was estimated as 98% by ^{1}H NMR.

Table 1 Abbreviations, structures and molecular weight (*M*) of the studied ILs.

Abbreviation	Structure	M_w/gmol^{-1}
[$C_1COOC_2OC_2$mIm][Tf_2N]		493
[$C_1COOC_2OC_2OC_4$mIm][Tf_2N]		566
[C_1COOC_5mIm][Tf_2N]		491
[C_1COOC_5mIm][C_8SO_4]		421
[C_6mIm][Tf_2N]		447
[C_8mIm][Tf_2N]		475

1-octanol (CAS N°111-87-5, Fluka, grade GC, purity>99.5%) and imidazole (CAS N° 288-32-4, Aldrich, purity 99%) were used without further purification. Distilled water was used for the physico-chemical determinations.

2.2. Aqueous solubility measurements

Aqueous solubility was determined following the OECD Guideline 105 [27]. A mixture of IL and distilled water was stirred for at least 72 hours at 27 °C and then stood for at least 24 hours at 22 °C. The aqueous solution was then diluted and its absorbance was measured with a UV-vis spectrophotometer (UVIKON 941) to determine the concentration of IL. We verified the thermodynamic equilibrium of the system by repeating the measurement after stirring the system one day more.

2.3. Octanol-water partition coefficients

The octanol-water partition coefficients of the different samples were determined

following the procedure described in the OECD Guideline 123 [19] at room temperature (22 ± 1 ℃). Both water and 1-octanol were mutually saturated prior to the experiment. Two stock bottles, one containing 1-octanol and a sufficient quantity of water, and the other containing water and a sufficient quantity of 1-octanol, were stirred using Teflon coated stir bar for at least two days, and then stand long enough to allow the phases to separate.

Depending on the detection limit and solubility of the IL in octanol, different low concentration solutions of the IL were prepared using 1-octanol pre-saturated with water as solvent. 5 ml water pre-saturated with 1-octanol were placed in a 7 ml glass vial with an open-top screw cap sealed with septum. 1.5 ml of the solution of IL in 1-octanol saturated with water was carefully added against the wall of vial, close to the water surface, reducing the possibility of formation of microdroplets from 1-octanol in the water phase. The vial was nearly filled for minimizing the vapour phase. It was closed tightly and stirred slowly using a Teflon coated stir bar for at least 48 hours, avoiding the vortex depth exceeding 1 cm. The two phases were allowed to separate for at least 2 hours. Each concentration was performed with 3 independent slow-stirring replicates. 1-octanol rich phases were withdrawn with all glass-metal syringes.

Concentrations of ILs in each phase were measured with a UV-vis spectrophotometer (*UVIKON 941*). Samples were diluted if their concentration exceeded the calibration range.

2.4. Diffusion coefficient Measurements

Diffusion coefficients were measured using the Taylor dispersion technique, using an apparatus previously described [22]. In this study, a Waters 600S pump was used to provide a constant flow rate of solvent (0.30 $mL.min^{-1}$) and a refractive index detector (Waters, model 2414) was used to detect the concentration profile of the solute. Before each measurement, the water was circulated at 2.00 $mL.min^{-1}$ for 20 minutes to make sure the apparatus was clean. The apparatus was calibrated by measuring the diffusion of potassium chloride in water at 298 K and tested using NaCl and methanol. The values obtained at 298K (1.55×10^{-9} $m^2.s^{-1}$ and 1.52×10^{-9} $m^2.s^{-1}$ respectively) are in good

agreement with the reported values of 1.52×10^{-9} $m^2.s^{-1}$ for NaCl [28] and 1.56×10^{-9} $m^2.s^{-1}$ for methanol [29].

2.5. Growth conditions and incubation with ILs

Four microbial strains were tested for their potential capacity to degrade a wide range of xenobiotics: three bacteria *Rhodococcus rhodochrous* ATCC 29672, *Pseudomonas viridiflava* strain 14b14, *Nocardia astero ïdes* strain 911 and the yeast *Candida parapsilosis* ATCC 20246. Nocardioforms [30], notably the genus *Nocardia*, and the genus *Rhodococcus* [31,32] have been described as potential degraders of aliphatic and aromatic hydrocarbons, long aliphatic chain (polyethylene), halogenated compounds, heterocyclic compounds and various herbicides. So far only Pseudomonads and related bacteria have been reported to possess comparable biodegradation abilities. *Pseudomonas* [33] are ubiquitous bacteria that can live under a wide range of environmental conditions and are characterized by their considerable catabolic versatility. *Candida* yeasts are known as models for alkane assimilation and have been widely used for metabolic studies as typical eukaryotic microorganisms.

Rhodococcus rhodochrous ATCC 29672 and *Pseudomonas viridiflava* strain 14B14 were grown in 100-mL portions of Trypcase-soy broth (bioMérieux, Marcy l'Etoile, France) in 500-mL Erlenmeyer flasks incubated at 27 ℃ and 200 rpm. The cells (300 mL of the culture medium) were harvested after 24 h of culture under sterile conditions and centrifuged at 8000 rpm for 15 min at 4 ℃. The bacterial pellet was washed first with a NaCl solution (8 $g.L^{-1}$) and then with Volvic® mineral water to keep a mineral composition constant. The resting cells (5×10^9 cells/mL) were incubated with 50 mL of the ionic liquid solution (1 $mmol.L^{-1}$in distilled water) in 250 mL Erlenmeyer flasks at 27 ℃ under agitation (200 rpm). Negative controls without cells (abiotic samples) or without substrate (cell blanks) were carried out under the same conditions. During the nine-week incubation, samples (1 mL) were taken regularly, centrifuged at 12,000 rpm for 5 min. The supernatants were immediately frozen and kept for subsequent NMR analysis.

The same procedure was followed for the yeast *Candida parapsilosis* ATCC 20246 and

the bacterium *Nocardia astero ïdes* strain 911 except that they were grown in Medium200 (ATCC) and Yeast Malt medium (For 1L: glucose 4g; yeast extract 4g; Malt extract 10g. The pH was adjusted to 7.4), respectively.

2.5.1. Cell counting

Ten fold serial dilutions were spread onto Trypcase-soy agar plates and incubated at 27 ℃ in the dark for at least 4 days, after which the number of colony forming unit (cfu) was counted in order to determine the viable number of cells.

2.5.2. Measurements of the nucleic acid contents

The absorbance (A_{260}) of supernatants was measured using a NanoDrop ND-100 spectrophotometer. None of the ILs tested absorbed at this wavelength.

2.5.3. ^{1}H Nuclear Magnetic Resonance (NMR) analyses.

The crude samples (540 µL) were supplemented with 60 µL of a 5 mmol.L^{-1} solution of tetradeuterated sodium trimethylsilylpropionate ($TSPd_4$, Eurisotop, Saint-Aubin, France) in D_2O. D_2O was used for locking and shimming. $TSPd_4$ constituted a reference for chemical shifts (0 ppm) and quantification.

^{1}H NMR was performed at 25 ℃ at 500 MHz on an Avance500 Bruker spectrometer (BrukerBiospin, Wissembourg, France) equipped with a triple-resonance (^{1}H, ^{13}C, ^{15}N) inverse probe with 5-mm-diameter tubes containing 600 µL of sample, with water being suppressed by a classical two phase-shifted pulse saturation sequence. 128 scans were collected (90 ° pulse, 7.3 µs; saturation pulse, 3 s; relaxation delay, 3 s; acquisition time, 4.679 s; 65,536 data points). A 1-Hz exponential line-broadening filter was applied before Fourier transformation, and a baseline correction was performed on spectra before integration using Bruker software (Topspin 2.0).

2.6. Toxicity

Five strains were tested to assess the antimicrobial activity of the ILs: four bacteria (*Bacillus cereus* ATCC 14579 and *Rhodococcus rhodochrous* ATCC 29672 as Gram positive bacteria, *Pseudomonas aeruginosa* ATCC 17504 and *Escherichia coli* ATCC

11303 as Gram negative ones) and one yeast (*Candida albicans* CIP444). The minimum inhibitory concentrations (MIC) were determined by serial two-fold dilutions using the conventional broth microdilution method. Inocula were prepared by growing the strains for 24 hours in Mueller Hinton (*B. cereus, E. coli* and *P. aeruginosa*), Trypcase-soy (*R. rhodochrous*) or Sabouraud (*C. albicans*) broths. The final inoculum density for the MIC determination was approximately 10^5 organisms/mL.

Uninoculated broth (100 µL) was dispensed in wells of the line A (sterile controls) of a 96 well microtitre plate whereas inoculated broth (100 µL) was dispended in the others wells except in column 1. A solution of the IL to be tested (150 µL) was added in the wells of column 1 (except in line A: sterile control and line H: growth control). 100 µL of the IL solution was added in column 2, mixed in the wells by drawing up and down a number of times and then 100 µL was transferred in the column 3. This twofold dilution was repeated down to column 12. The microtitre plates were incubated at 27 °C (37 °C for *E. coli*) for 24 to 48 hours. The MIC was the lowest concentration of IL inhibiting visible growth of the microorganisms. The MIC determinations were performed in triplicate.

The starting concentrations tested depend on the initial dissolution of the IL, considered as the maximum concentration of ILs soluble in water after stirring less than 20 minutes, varying from 40.6 mmol.L^{-1} for the most soluble ($[C_1COOC_2OC_2mIm][Tf_2N]$) to 2.8 mmol.$L^{-1}$ for the less soluble one ($[C_8mIm][Tf_2N]$). All the initial concentrations were checked by ^{1}H NMR.

3. Results and Discussion

3.1. Solubility in water

The experimental values of the aqueous solubility are listed in Table 2. This property for the oxygenated $[Tf_2N]$ based ILs ranges from 25 to 63 mmol.L^{-1}. It is nearly one order of magnitude higher than the aqueous solubility of Tf_2N based ILs without oxygen groups (Table 2). The incorporation of oxygen groups in the apolar alkyl chain of the cation enhances the polarity of solute and as a consequence the aqueous solubility is higher. An increase of the alkyl chain length on the imidazolium ring (lower polarity) leads to a

decrease of the aqueous solubility. This was also observed in literature in the case of non-functionalized imidazolium-based ILs [34,35]. The anion [C_8SO_4] makes the IL totally miscible with water.

Table 2 Aqueous solubility (AS) both in mmol.L^{-1} and mole fraction (x) of functionalized ILs at 22 ±1 ℃ (this work) or non-functionalized ILs at 23.5 ±1 ℃ [34].

ILS	AS	
	mmol.L^{-1}	$x \times 10^4$
[$C_1COOC_2OC_2mIm$][Tf_2N]	44 ±5	7.9 ±0.9
[$C_1COOC_2OC_2OC_4mIm$][Tf_2N]	25 ±3	4.5 ±0.5
[C_1COOC_5mIm][Tf_2N]	63 ±7	11 ±1
[C_1COOC_5mIm][C_8SO_4]	Miscible	
[C_4mIm][Tf_2N]	17 ±7 [34]	3.1 ±1.3
[C_6mIm][Tf_2N]	5.1 ±0.9 [34]	0.9 ±0.1
[C_8mIm][Tf_2N]	4.0 ±0.7 [34]	0.7 ±0.1

It has been evidenced during the aqueous solubility determinations, that the imidazolium cations with an ester function react with water giving an hydrolysis product – the 3-methyl-1-(carboxymethyl)imidazolium ([$C_1COOHmIm$]) – totally miscible with water. This product is also detected by UV absorption. Given the extinction coefficients and wavelengths of maximum absorption of the ILs and of the hydrolysis product, it was impossible to estimate the contribution of each component separately. The equilibrium concentration in water (aqueous solubility) is in fact a global concentration, at equilibrium of both the IL and its hydrolysis product. Kinetic studies were monitored by ^1H NMR and showed that the percentage of the hydrolysis product was less than 10% under the conditions used to determine aqueous solubility.

3.2. Octanol-water partition coefficients

In order to test the method for measuring the octanol-water partition coefficients, the K_{OW} for imidazole ($C_3H_4N_2$) was measured and the value obtained was compared with the

literature. We have obtained a value for K_{OW} of 0.90 ± 0.09, compared with the literature data of 0.83 [18]. The deviation of 8% is considered adequate for this type of experimental determinations [20].

The partition coefficient was measured at different IL concentrations in the aqueous phase, C_{IL}, fitted as a function of this concentration and extrapolated at infinite dilution. All the data are presented in Table 3. A slight increase of the partition coefficient with concentration was observed despite the IL concentration being as low as 10^{-3} mol.L^{-1}. This was also observed in literature using direct measurement methods [21,36,37].

Due to this variation and the uncertainty of the fitting, a precision of 10 to 30 % was calculated on the partition coefficient. These high values can be explained by the difficulty of the measurement often attributed to the possibility of forming invisible micro droplets of octanol in water-rich phase.

As shown in Table 3, the partition coefficient of ILs with oxygenated functions varies from 0.01 to 1.18 (log *Kow* from -2 to 0.07). These values are far below the bioaccumulation limit, thus the considered ILs are not very hydrophobic.

Although K_{OW} is a key parameter to characterise the environmental impact, it has been rarely investigated for ILs. Data of literature for imidazolium-based ILs are presented in Table 4. They are obtained by experimental techniques or predicted using different empirical or semi-empirical methods. Big disparities between different sets of data confirm the difficulty of measuring or predicting this property. Nevertheless, as shown in Table 4, the same order of magnitude was obtained for the octanol-water partition coefficient in ILs with or without oxygen functionalization.

Table 3 Experimental octanol-water partition coefficients, K_{ow} at different IL concentrations, C_{IL} and at infinite dilution, K_{OW}^{∞}, at 22 ℃.

IL	$\frac{C_{IL}}{mM}$	K_{OW}	IL	$\frac{C_{IL}}{mM}$	K_{OW}
$[C_1COOC_2OC_2mIm][Tf_2N]$	1.13	0.06	$[C_1COOC_5mIm][Tf_2N]$	0.18	1.37
K_{OW}^{∞} = 0.01	1.18	0.03	K_{OW}^{∞} = 1.18	0.19	1.30
	1.20	0.06		0.23	1.09
	2.03	0.06		0.34	1.38
	2.21	0.06		0.37	1.10
	2.28	0.05		0.57	1.15
	2.70	0.09		0.60	1.40
	2.84	0.08		0.62	1.39
	2.99	0.10		0.71	1.57
	3.16	0.11		0.89	1.65
	3.37	0.13		0.91	1.62
	3.40	0.15		1.00	1.47
	3.41	0.11		1.09	1.41
	3.41	0.15		1.17	1.73
	3.58	0.12		1.45	1.45
				1.46	1.36
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	0.69	0.26	$[C_1COOC_5mIm][C_8SO_4]$	0.37	0.53
K_{OW}^{∞} = 0.11	0.83	0.35	K_{OW}^{∞} = 0.57	0.38	0.49
	0.91	0.31		0.52	0.93
	1.25	0.39		0.53	0.89
	1.38	0.40		0.64	1.16
	1.47	0.45		0.68	1.02
	1.68	0.51		0.74	1.61
	1.87	0.55		0.77	1.28
	1.87	0.56		0.77	1.28
	1.97	0.57		0.87	1.25
	2.21	0.65			
	2.27	0.67			
	2.30	0.55			
	2.34	0.75			
	2.45	0.65			
$[C_1COOH$ mIm][Cl]		<0.01			

Table 4 Octanol-water partition coefficients, K_{OW}, of 3-methyl-1-alkylimidazolium, [C_nmIm]-based ILs and of some organic compounds between 20 ℃ – 30 ℃ from literature.

Compounds	K_{OW}	Compounds	K_{OW}
[C_2mIm][Tf_2N]	0.09-0.11 [21]	[C_3mIm][BF_4]	0.0182 [38]
[C_4mIm][Tf_2N]	0.11-0.62 [21] 0.02-3.16 [37]	[C_4mIm][BF_4]	0.0363 [38], 0.0030 [21]
[C_6mIm][Tf_2N]	1.42-1.66 [21]	[C_5mIm][BF_4]	0.0813 [38]
[C_8mIm][Tf_2N]	6.3-11.1 [21]	[C_6mIm][BF_4]	0.1950 [38]
[C_4mIm][Cl]	0.0040 [21]; 0.48 [35]	[C_2mIm][PF_6]	0.015 [36]
[C_8mIm][Cl]	0.54 [34]	[C_4mIm][PF_6]	0.02 [36]; 0.022 [21]; 0.004-0.1 [37]
[C_{10}mIm][Cl]	0.52 [35]	[C_4mIm][NO_3]	0.0038 [21]
[C_{12}mIm][Cl]	0.73 [35]	[C_4mIm][Br]	0.0033 [21]
[C_2mIm][$B(CN)_4$]	0.169 [34]		
C_2H_5OH	0.57 [20]	$Cl_2C=CH_2$	123 [20]
$(CH_3)_2CO$	0.50 [20]	$Cl_2C=CHCl$	263 [20]
1-C_4H_9OH	6.92 [20]	C_6Cl_6	275423 [20]

[21] slow-stirring method; [36,37] shake-flask method; [34,35] indirect method; [38] calculation method.

Imidazolium-based-ILs without oxygen functionalization and sharing the same anion present K_{OW} that increases as the alkyl side chain on the cation increases. For the oxygen functionalized ILs, [$C_1COOC_2OC_2$mIm][Tf_2N] has the lowest K_{OW} value and [C_1COOC_5mIm][Tf_2N] the largest. The values increase with the length of the alkyl chain of the imidazolium cation, beside the ether/ester function, not with the total length of the side chain of the imidazolium ring. For ILs with an equivalent number of carbon atoms (total carbon atoms in the case of non-functionalized ILs, carbons beside the oxygenated function in the case of modified ILs), the K_{OW} values of the ILs pairs [$C_1COOC_2OC_2$mIm][Tf_2N] and [C_2mIm][Tf_2N], [$C_1COOC_2OC_2OC_4$mIm][Tf_2N] and [C_4mIm][Tf_2N], [C_1COOC_5mIm][Tf_2N] and [C_6mIm][Tf_2N], are of the same order of magnitude, but slightly lower in the presence of oxygen. The long apolar alkyl chains lead to higher K_{OW} values, while the presence of oxygen can break this effect. This result

confirms the empirical statement of Soskic and Plavsic [39] who claim that the contributions to K_{OW} of heteroatom in the alkyl chains of the ions are usually negative.

Compared to some widely used chemicals (Table 4), K_{OW} values for ILs are similar to those of low-molecular weight polar solvents (except halogenated solvents), much lower than those of high molecular weight polar solvents and apolar solvents. From the analysis of K_{OW}, ILs seem to present a lower environmental impact than many traditional organic solvents.

The K_{OW} values of all the considered methylimidazolium-based ILs indicate they are not lipophilic and thus have low potential to accumulate or concentrate in the environment and in living organism. They have low rate of passive diffusion across a biological membrane, low possibility of being stored in fat depots or in other lipophilic sites such as membranes or lipoproteins.

The octanol-water partition coefficient of [C_1COOHmIm][Cl] (hydrolysis product of the IL containing ester group) was also measured in order to estimate the environmental impact of a degradation product. When C_{IL} is in the required range - less than 10 mmol.L^{-1} -, the concentration at equilibrium in the aqueous phase is below our detection limit. We can thus conclude that the K_{OW} value of [C_1COOHmIm][Cl] is less than 0.01, which is in favour of a minor environmental impact.

3.3. Diffusion coefficients

The average values of diffusion coefficients for ILs in water at infinite dilution (D_{AB}) are listed in Table 5. The overall scatter of the data points gives a precision of 0.02 $\times$ 10^{-9} $m^2.s^{-1}$, corresponding to a (1 to 2) % relative error associated with the data. The total uncertainty of the diffusion coefficients is evaluated to be 5% by simple error propagation analysis taking into account the various parameters of the experimental method.

The diffusion coefficients for all the ILs are in the same order of magnitude, varying from 0.74 to 0.96 $\times$ 10^{-9} $m^2.s^{-1}$ at 303 K with a decrease, as expected, of this coefficient with the length of the alkyl chain on the cation. Compared to the non-functionalised ILs [22] (Table 5), the presence of an oxygenated function in the IL, for a given alkyl chain length, leads to

a decrease of the diffusion coefficient. The diffusion coefficient of ILs in water is comparable to the diffusion coefficient of low molecular weight alcohols in water (ethanol, 0.84×10^{-9} $m^2.s^{-1}$; n-butanol, 0.77×10^{-9} $m^2.s^{-1}$ [40]. The experimental data were fitted to Arrhenius plots of the form:

$$\ln(D_{AB}) = \ln D_{\infty} + \frac{E_a}{RT} \qquad (1)$$

where D_{∞} represents the diffusion coefficient at infinite temperature, and E_a the activation energy of the solute for diffusion. The values for E_a and D_{∞} with the relative standard deviation (σ) are also listed in Table 5.

Table 5 Experimental diffusion coefficients, D_{AB}, for ILs in water at infinite dilution and parameters (E_a and D_0) for fitting eq. (1) from 283 K to 303 K along with standard deviation $\sigma. \sigma = \sum \left| D_{AB}^{\exp} - D_{AB}^{cal} \right| / {}_{AB}^{\exp} / n$

	$D_{AB}/10^{-9}m^2.s^{-1}$			E_a	D_{∞}	σ
	283 K	293 K	303 K	$kJmol^{-1}$	$10^{-9}m^2s^{-1}$	
$[C_1COOC_2OC_2mIm][Tf_2N]$	0.48	0.65	0.85	-20.4	2788	0.3%
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	0.43	0.57	0.74	-19.4	1612	0.1%
$[C_1COOC_5mIm][Tf_2N]$	0.49	0.66	0.86	-20.1	2484	0.3%
$[C_1COOC_5mIm][C_8SO_4]$	0.45	0.59	0.77	-19.2	1539	0.3%
$[C_4mIm][Tf_2N]$[22]	0.60	-	0.96	-16.8	752.1	
$[C_6mIm][Tf_2N]$[22]	0.58	-	0.90	-16.4	612.4	
$[C_8mIm][Tf_2N]$[22]	0.57	-	0.87	-15.0	342.1	

Wilke–Chang equation is commonly used for estimating diffusion coefficient of organic molecules (A) in a solvent (B) [22-24] as a function of temperature:

$$D_{AB}(T) = 7.4 \times 10^{-12} \frac{(\psi M_B)^{0.5} T}{\eta_B V_A^{0.6}} \qquad (2)$$

where ψ is the association parameter for the solvent (water = 2.26 [23]). M_B and η_B are the molar mass and viscosity of solvent in $g.mol^{-1}$ and in mPa.s. The viscosity values of water

at 283.15 K, 293.15 K, 303.15 K are 1.307, 1.002 and 0.7975 mPa.s, respectively [41]. V_A is the molar volume of the solute in cm^3 mol^{-1} at its normal boiling point. As in other studies [22-24], the Wilke–Chang equation underestimated the diffusion coefficient values in the present work, hence we correct the equation.

The original form of this estimation method requires the knowledge of the experimental volumetric property. For the purpose of developing a predictive model without any input of experimental data, V_A can be replaced by the Van der Waals volume (V_{vdw}) , calculated using a simple model [42]:

$$V_{vdW} = \sum V_{vdW}^{atom} - 14.7R_A - 3.8R_{NA} - 5.92N_B \qquad (3)$$

where V_{vdW}^{atom} is the van der Waals volume of each atoms [42] in the molecule (V_{vdW}^{atom} should be 6.04 instead of 40.48 reported in the paper), R_A is the number of aromatic rings, and R_{NA} is the number of non-aromatic rings. N_B is the number of bonds in a molecule, $N_B = N - 1 + R_g$. where N is the total number of atoms and R_g is the total number of ring structures ($R_g = R_A + R_{NA}$).

Then the original empirical coefficient is corrected by training our data together with the actual available data of 19 non-functionalized imidazolium-based ILs with different anions reported in literature [22-24], and the equation to predict the diffusion coefficient as a function of temperature is finally:

$$D_{AB}(T) = 8\times10^{-11}\frac{T}{\eta_B V_{vdw}^{0.6}} \qquad (4)$$

The predictive capacity of this equation is satisfactory to an average prediction error of less than 5%. But for several ILs based on other anions, for example the predict error of $[C_2mIm][C_2SO_4]$ [23] is 27%, which probably suggests that the ILs with different anions need different equations, meaning more experimental data are needed for training**.**

3.4. Biodegradation of the ILs studied

The biodegradation kinetics of each IL (1 $mmol.L^{-1}$ in water) were monitored in resting-cell incubation experiments for each strain tested (*Rhodococcus rhodochrous*

ATCC 29672, *Pseudomonas viridiflava* strain 14b14, *Nocardia astero ïdes* strain 911 and the yeast *Candida parapsilosis* ATCC 20246) and compared with incubation under abiotic conditions. Samples were taken regularly during a nine-week period and analysed by ^{1}H NMR. As the results obtained with the 4 strains tested were very similar, we have chosen to present in detail and illustrate the ones obtained with *Rhodococcus rhodochrous* ATCC 29672, the intrinsic metabolites present in the medium remaining in traces with this strain within the incubation period conversely to the others (cell blanks).

3.4.1. Biodegradation of the long alkyl chain ILs

The long alkyl chain ILs, [C_6mIm] and [C_8mIm], were not degraded either under abiotic or biotic conditions (Fig. 1).

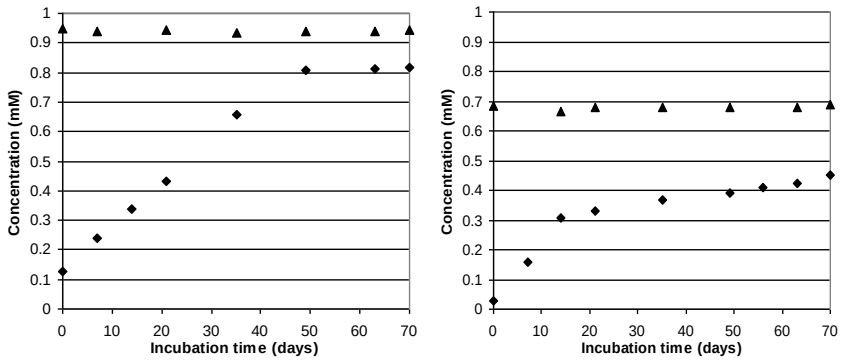


Fig. 1 Kinetics of [C_6mIm][Tf_2N] (left plot) and [C_8mIm][Tf_2N] (right plot) incubation under abiotic (▲) and biotic conditions (with *Rhodococcus rhodochrous* ATCC 29672) (◆). Mean values from 3 independent experiments.

It is worth noting that the IL concentration increased with time in the presence of microorganisms, reaching the expected initial concentration only after several weeks. Adsorption or strong interactions with the cells can be suggested to explain this result. The evolution of the ^{1}H NMR spectra recorded at different times of incubation can be significantly interpreted. The appearance of many new intense signals was observed in the presence of both ILs (in particular with [C_8mIm]), that are not present in the negative control with cells alone (Fig. 2). This class of ILs has been shown previously to act as detergents [43] and probably lead to cell lysis.

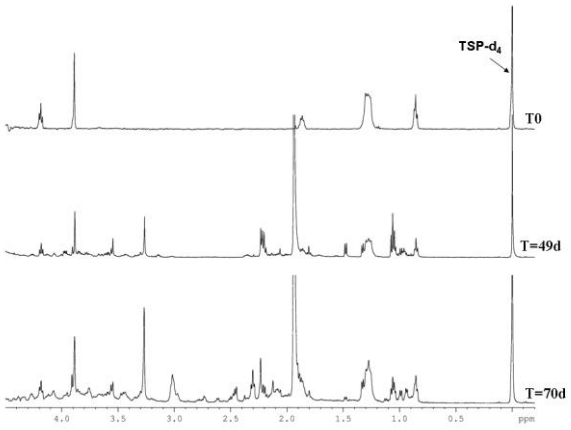


Fig. 2 ^{1}H NMR spectra (aliphatic region) recorded during the biodegradation of [C_8mIm][Tf_2N] by *Rhodococcus rhodochrous* ATCC 29672 after T0, T49 and T70 days of incubation.

To further investigate the effect of long alkyl chain ILs on cells compared with the oxygen-functionalised ILs, the evolution of the cell number was estimated by cell counting on agar plate of incubation samples taken at different times in the negative cell control and in the presence of [C_8mIm], [C_1COOC_5mIm][Tf_2N] and [C_1COOC_5mIm][C_8SO_4]. A measurement of the nucleic acid contents in the supernatants of the same samples was also carried out. The results are presented in Fig. 3.

A marked decrease of the cell number was observed in the case of [C_8mIm] getting with a great increase of the nucleic acid amount present in the medium. No such observation was made in the negative control containing cells alone where these data were relatively stable during the 50-day monitoring.

These results confirm the cell lysis due to the detergent effect of [C_8mIm] on the cell walls leading to the release of intracellular compounds into the medium (Fig. 2). The [C_8mIm] itself is then slowly released into the medium as the cell walls are broken (Fig. 1). This effect on the membrane integrity has also recently been clearly evidenced on rat pheochromocytoma cells for even shorter alkyl chain length ([C_4mIm]) by measurement of lactate dehydrogenase release [44]. It was not observed for [C_1COOC_5mIm][Tf_2N] and [C_1COOC_5mIm][C_8SO_4].

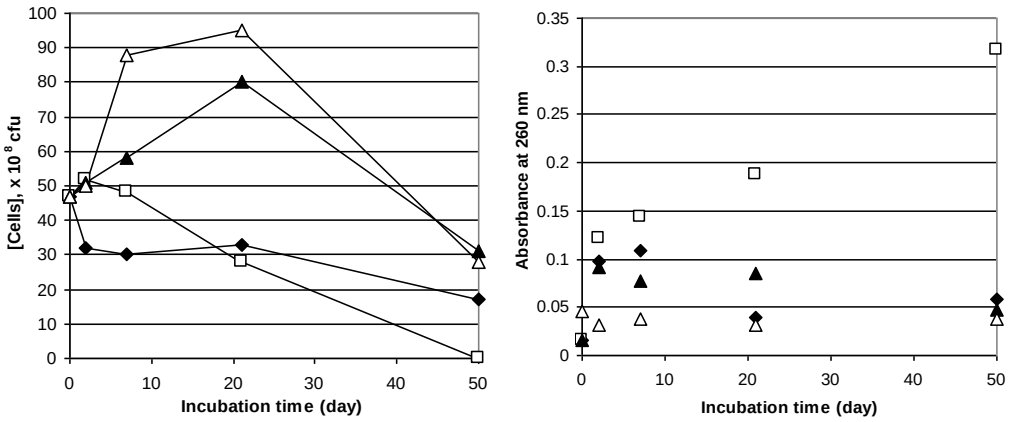


Fig. 3 Evolution of the cell number (left plot) and of the nucleic acid content (right plot) in the supernatant within the 50-day incubation with *Rhodococcus rhodochrous* ATCC 29672. Negative control (◆); $[C_8mIm][Tf_2N]$ (□); $[C_1COOC_5mIm][Tf_2N]$ (▲) and $[C_1COOC_5mIm][C_8SO_4]$ (△).

3.4.2. Biodegradation of the oxygenated-functionalised ILs

The four ester-functionalised ILs tested were readily transformed under abiotic and biotic conditions. A major common metabolite was formed quantitatively as monitored by ^1H NMR and accumulated in the medium whatever the structure of the starting IL and the conditions. This compound was easily identified by LC-MS (m/z = 141.0669) and ^1H NMR as the hydrolysis product $[C_1COOHmIm]$ (Fig. 4). The chemical structure of this compound was confirmed by its synthesis and its co-analysis by ^1H NMR with a sample obtained during the microbial incubation. Under biotic conditions, the pentanol released after hydrolysis of $[C_1COOC_5mIm][Tf_2N]$ and $[C_1COOC_5mIm][C_8SO_4]$ was detected only in the samples taken at short incubation times and degraded rapidly. In the case of $[C_1COOC_2OC_2OC_4mIm][Tf_2N]$ and $[C_1COOC_2OC_2mIm][Tf_2N]$, the alcohols with an ether alkyl chain released were rapidly oxidized into carboxylic acids that accumulate in the medium (Fig. 4).

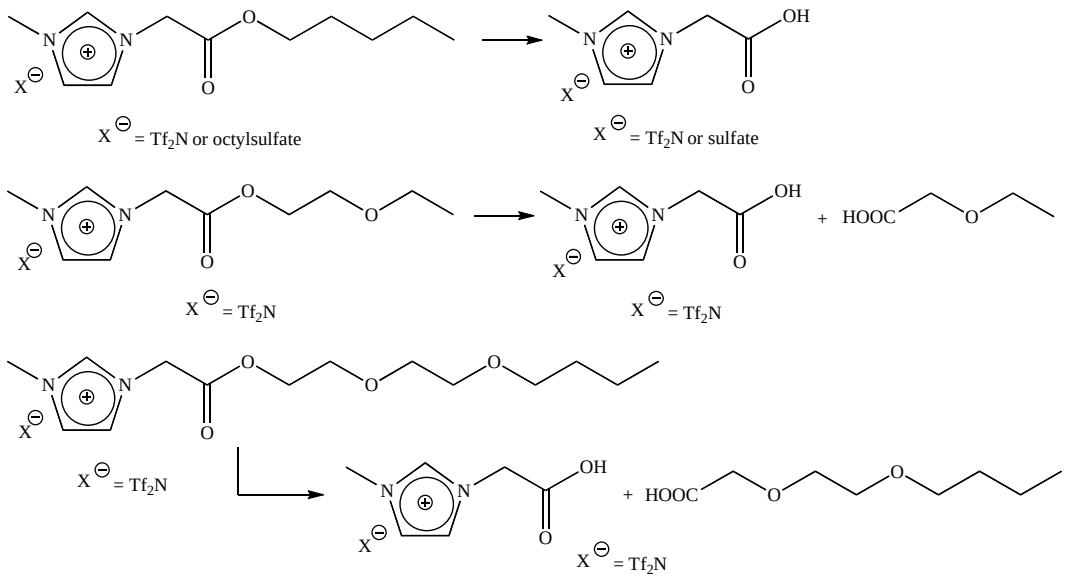


Fig. 4 Metabolic pathways of the studied ILs.

The rate of the hydrolysis reaction kinetics, considered as first-order kinetics, was greatly enhanced in the presence of microorganism and the reaction was complete, proving the role of biological activity (Table 6).

Table 6 Kinetics parameters of IL hydrolysis under abiotic and biotic (*Rhodococcusrhodochrous* ATCC 29672) conditions. Mean values of 2 or 3 independent experiments.

ILs	Abiotic conditions			Biotic conditions		
	K (day^{-1})	r	%hyd. after 70 days	K (h^{-1})	r	% hyd. (hours)
$[C_1COOC_5mIm][Tf_2N]$	0.0098	0.997	51%	0.78	0.994	100% (4.5)
$[C_1COOC_5mIm][C_8SO_4]$	0.0096	0.977	53%	0.18	0.989	100% (12.5)
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	0.032	0.998	89%	0.37	0.975	100% (23)
$[C_1COOC_2OC_2mIm][Tf_2N]$	0.030	0.991	81%	0.295	0.981	100% (23)

The nature of the anion (octylsulfate versus Tf_2N) had a marked effect on the global biodegradation rate (decrease by a factor > 4 with the octylsulfate anion) (upper plot in Fig. 5). The monitoring of the octylsulfate concentration in the case of $[C_1COOC_5mIm][C_8SO_4]$

by ^{1}H NMR showed its disappearance only in the presence of microorganism. It was completely degraded within 5 days (lower plot in Fig. 5). This IL was found as "readily biodegradable" with the "CO_2 headspace test" [10] contrary to $[C_1COOC_5mIm][Tf_2N]$, although in our case, the final product is the same and it is biotransformed less rapidly.

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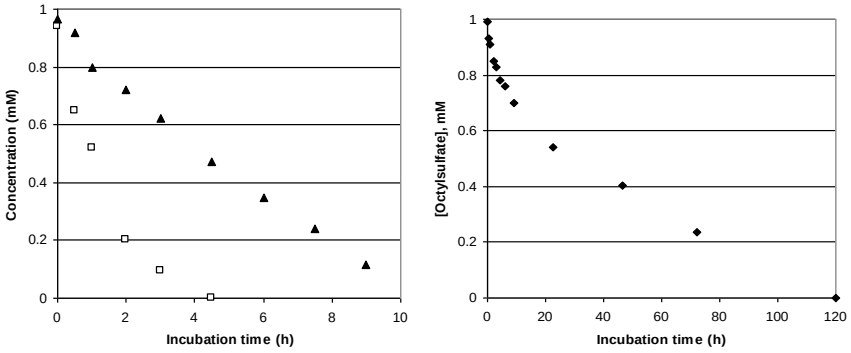

Fig. 5 Biodegradation kinetics by *Rhodococcus rhodochrous* of: in the left plot $[C_1COOC_5mIm][Tf_2N]$(□) and $[C_1COOC_5mIm][C_8SO_4]$ (▲); in the right plot, octylsulfate anion. Mean values from 3 independent experiments

For the same chain length, the introduction of an ethereal moiety has also a negative effect on the global biodegradation rate (comparison of $[C_1COOC_5mIm][Tf_2N]$ and $[C_1COOC_2OC_2mIm][Tf_2N]$ in Table 6) but favours its hydrolysis under abiotic conditions.

The same metabolic pathways, as well as similar biodegradation rates, were obtained with the three other strains tested: *Pseudomonas viridiflava* strain 14b14, *Nocardiaastero ïdes* strain 911 and the yeast *Candida parapsilosis* ATCC 20246. This study demonstrates that the imidazolium ring was not degraded by the strains tested. This moiety has already been described as a factor of limiting biodegradability [43]. The introduction of oxygen-functionalized side chains (ester) increases the biodegradability but leads to the accumulation of metabolites, in particular via hydrolysis reaction. This reaction that also occurs under abiotic conditions even with a slower rate can affect the performance of these ILs from a synthetic point of view. The presence of ethereal side chains was rather a disadvantage for biodegradation.

3.5. Toxicity studies

Five strains were tested to assess the antimicrobial activity of the ILs: four bacteria (*Bacillus cereus* ATCC 14579 and *Rhodococcus rhodochrous* ATCC 29672 as Gram positive bacteria, *Pseudomonas aeruginosa* ATCC 17504 and *Escherichia coli* ATCC 11303 as Gram negative bacteria) and one yeast (*Candida albicans* CIP 444). The ILs tested present a wide range of solubility in water as shown previously. In order to avoid the effect of solvent or/and IL precipitation in the medium after solvent evaporation, the choice was made to adapt the starting IL concentration for MIC evaluation in function of its initial dissolution. The supposed initial IL concentration was checked by NMR. The minimum inhibitory concentrations (MIC) of ILs are listed in Table 7.

Table 7 Minimum inhibitory concentrations (MIC) of ILs ($mmol.L^{-1}$).*B.c. Bacillus cereus ATCC 14579; P. a. Pseudomonas aeruginosa ATCC 17504; E. c. Escherichia coli ATCC 11303; R. r. Rhodococcus rhodochrous ATCC 29672; C. a. Candida albicans CIP 444.*

	B. c.	*P.a*	*E. c*	*R. r.*	*C. a.*
$[C_6mIm][Tf_2N]$	2	> 2	1	1	> 2
$[C_8mIm][Tf_2N]$	1.4	> 1.4	0.7	0.3	1.4
$[C_1COOC_2OC_2mIm][Tf_2N]$	20.3	> 20.3	10.1	10.1	> 20.3
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	> 6.4	> 6.4	6.4	6.4	> 6.4
$[C_1COOC_5mIm][Tf_2N]$	> 5.5	> 5.5	5.5	5.5	> 5.5
$[C_1COOC_5mIm][C_8SO_4]$	3.8	7.5	3.8	7.5	3.8
$[C_1COOHmIm][Cl]$	> 23	> 23	> 23	> 23	> 23

According to the results, the oxygen-functionalised ILs are not very toxic for the strains tested. In most cases, their MIC is superior to their solubility. The presence of oxygen in the alkyl chains reduced the toxicity of ILs. It is worth noting that in this study, the anion $[C_8SO_4^-]$ slightly increases the toxicity compared with $[Tf_2N^-]$. This result differs from the previous study of Morrissey et al. [45]. For the ILs with $[Tf_2N^-]$, similarly with K_{OW}, $[C_1COOC_5mIm][Tf_2N]$ is the most toxic whereas $[C_1COOC_2OC_2mIm][Tf_2N]$ presents the lowest MIC value. The toxicity increases with the length of apolar alkyl chain, beside the

ether/ester function, not with the total length of the side chain of the imidazolium ring.

Differences were observed between strains: *E. coli* is the most sensitive and *P. aeruginosa* is the most resistant. A similar conclusion was obtained by Morrissey et al. [45] with 1-methyl-3-(decyloxycarbonyl)methylimidazolium bromide.

After 24 or 48 hours of incubation, the final IL concentration was checked in each case and the hydrolysis percentage was determined. According to the strain and the IL structure, the hydrolysis product was more or less present. Its toxicity was therefore also tested and showed no important biological activity. Nevertheless, the results obtained with the hydrolysable ILs can be distorted by its percentage of presence, giving rather a lower toxicity to the starting IL.

4. Conclusions

The aim of this work was to assess the consequences on the environmental impact of the presence of oxygenated moities (ester and ether groups) on the alkyl chain of imidazolium-based ILs. A two fold strategy was followed with on one hand the determination of pertinent physico-chemical properties like the water solubility, the diffusivity in water and the octanol-water partition coefficient and, on the other hand, the evaluation of the toxicity and the biodegradability of the selected ILs

The presence of both ester and ether groups on the 1-alkyl-3-methylimidazolium cation decreases the value of their octanol-water partition coefficient, which means that the lipophilicity of this family of ILs decreases when the alkyl side-chains contain these oxygen functionalities, and simultaneously reduces the toxicity of ionic liquids with $[Tf_2N]$ or $[C_8SO_4]$ anions. The known relationship between the toxicity and the value of K_{OW} for different chemicals is confirmed also for the families of ionic liquids studied herein. Further work is required in order to quantify this relationship and to use the octanol-water partition coefficient to estimate the toxicity of ILs.

For $[C_nmIm][Tf_2N]$ ionic liquids like the ones studied in this work, we can conclude that the presence of an ester function in the side chain renders the IL more easily biodegradable. Nevertheless these ester functions make the ionic liquid more susceptible to hydrolysis under abiotic and biotic conditions leading to the formation of chemical species

that are more difficult to biodegrade by the strains used in the present work. A careful study of the degradation products indicates a resistance of the imidazolium ring to biodegradability and to abiotic degradation. The presence of side-chains with the ether function was rather a disadvantage for biodegradation.

The modification of the structure of the ILs also changes some of the physico-chemical properties relevant to determine their environmental impact. The oxygen functionalised ILs are more soluble in water and, because they are heavier and larger than the non-functionalised equivalent, diffuse more slowly in this medium. These two properties will surely affect their transport and transfer in the environmental compartments, namely in aqueous environment.

Acknowledgement

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Physico-chemical Properties of Oxygen-functionalized Ionic Liquids - Density, Viscosity, and Carbon Dioxide Solubility as a Function of Temperature

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ABSTRACT

The physico-chemical properties (density, viscosity, and carbon dioxide solubility) of ionic liquids based on pyridinium, pyrrolidinium and ammonium cations were studied at atmospheric pressure and as a function of temperature between 293 and 343 K. The influence of the inclusion of oxygen functional groups (hydroxyl and ester) in the cations was assessed by comparing their behavior with that of similar non-functionalized ionic liquids. We observed that the presence of oxygen groups does not affect the density significantly. The inclusion of an ester group in the alkyl-side chain of pyridinium or ammonium cations greatly increases the viscosity of bis(trifluoromethylsulfonyl)imide ionic liquids while the presence of hydroxyl groups only slightly increases their viscosity. Carbon dioxide solubilities are not significantly influenced by the introduction of oxygen functional groups in the cations for the ammonium-based ionic liquids. In the case of the pyridinium-based ionic liquids, the solubility of carbon dioxide significantly decreases due to a defavourable entropic contribution to the Gibbs energy of solvation.

KEYWORDS ionic liquids, hydroxyl group, ester group, density, viscosity, carbon dioxide solubility.

INTRODUCTION

Ionic liquids (ILs) are candidates to substitute the classic volatile organic solvents, due to their interesting properties. Recent researches have demonstrated that the ILs can be toxic[1] and resist biodegradation[2]. It has been reported that the introduction of ester or ester plus ether groups, which are susceptible to enzymatic hydrolysis, in the alkyl side chains of imidazolim and pyridinium-based ionic liquids also can greatly improve their biodegradability.[2]

The chemical modification introduced to increase the biodegradability of these components will surely modify their physico-chemical properties. So far, there is a lack of information about the physico-chemical properties of the ester and/or ether functionalized ILs. Therefore, prior to considering these ILs for further studies or applications, a systematic and sufficiently accurate data set of physico-chemical properties is required. Understanding the relationship between the molecular structures and properties is essential for pursuing the aim of designing and synthesising ILs possessing interesting properties as well as low environmental impact.

Density and viscosity are relevant properties for the development of chemical processes, being related to mass transfer and power requirements for stirring.[3] The insertion of ether groups in the alkyl chain on both cation and anion has been shown to decrease the viscosities of ionic liquids[4], an observation that has been explained by an increasing flexibility of the functionalized alkyl chains and by the interactions between the oxygen atoms and the positively charged imidazolium core.

The influence of oxygen functional groups on CO_2 solubility has been previously studied. Carbonyl groups are expected to be CO_2-philes, because the oxygen is, in principle, able to take part in specific molecular interactions with CO_2.[5] Nevertheless, it has been proven, both experimentally[6,7] and by molecular simulation[8], that ester groups do not introduce a significant change in the carbon dioxide or ethane solubility. The presence of ether functionalities is also expected to affect favourably the carbon dioxide solubility, because it increases the free volume and the oxygen interacts with the carbon of CO_2.[5] Almost no differences in solubility were detected experimentally both in imidazolium-based ionic liquids (with bis(trifluoromethylsulfonyl)imide)[7] and in ammonium-based ionic liquids

(with alkylsulfate anions).[6]

The large majority of the ionic liquids with oxygen functionalities studied up to now are based in alkyl imidazolium cations. Herein, we have decided to study the density, the viscosity, and the carbon dioxide solubility of bis(trifluoromethylsulfonyl)imide, [Tf_2N], ILs associated to pyridinium, pyrrolidinium or ammonium cations with alkyl-side chains on the cation non-functionalized or having hydroxyl or ester moieties. Because our aim is to understand the effect of the functionalization of the physico-chemical properties of several families of ionic liquids, we have chosen cations with aromatic rings, aliphatic rings and non cyclic. The anion [Tf_2N] was chosen because it contributes to the reduction of the viscosity of the ILs[3] and increases their capacity to dissolve CO_2[9].

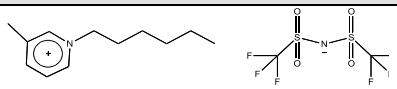
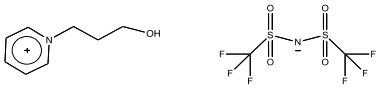
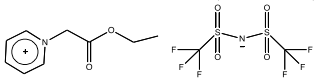
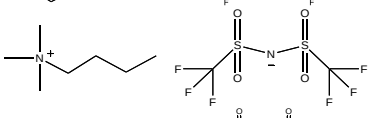
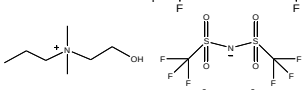
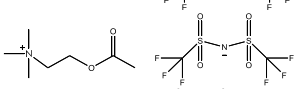
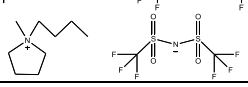
EXPERIMENTAL

Materials

The structures and abbreviations of the ILs used in this study are listed in Table 1. Three pyridinium-based ILs ([C_3Py][Tf_2N], [C_1COOC_2Py][Tf_2N], [C_3OHPy][Tf_2N]) and one pyrrolidinium-based IL ([C_6mPyr][Tf_2N]) were purchased from Iolitec (Germany), their purity being claimed to be higher than 98%. One ammonium-based IL ([$N_{1112OOCCH3}$][Tf_2N]) was purchased from Solvionic (France) with a stated purity higher than 98%. The other ILs ([C_6mPy][Tf_2N], [C_4mPyr][Tf_2N], [N_{4111}][Tf_2N] and [N_{1132OH}][Tf_2N]) were supplied by Queen's University of Belfast, UK. Their purity is above 99% as checked by ^1H NMR.

All the samples were dried and degassed under vacuum (pressure lower than 1 Pa) for at least ten hours before use. Their water content was measured by coulometric Karl Fisher titration (model Mettler Toledo DL32, standard method KF) and was found to be less than 150 ppm (w/w) in all samples.

Table 1. Abbreviation, structures, cation names and molecular weight (M) of the studied ionic liquids

Abbreviation	Structure	Name of cation
$[C_6mPy][Tf_2N]$		1-hexyl-3-methylpyridinium
$[C_3OHPy][Tf_2N]$		1-(3-hydroxypropyl)pyridinium
$[C_1COOC_2Py][Tf_2N]$		1-(2-ethoxycarbonyl)methylpyridinium
$[N_{4111}][Tf_2N]$		Trimethylbutylammonium
$[N_{1132OH}][Tf_2N]$		2-hydroxyethyl-dimethylpropyl ammonium
$[N_{1112OOCCH3}][Tf_2N]$		2-acetoxyethyl-trimethylammonium
$[C_4mPyr][Tf_2N]$		1,1-butyl-methylpyrrolidinium

Density measurements

The densities (ρ) of the samples at atmosphere pressure were measured using a U-shape vibrating tube densimeter (Anton Paar DMA 512P) as a function of temperature between 293 and 343 K in steps of 10K with a precision better than 0.01%.[7] The calibration of the densimeter was done using n-heptane, bromobenzene, and 2,4-dichlorotoluene, as reference fluids as described in detail in a previous publication.[10]

Viscosity measurements

The dynamic viscosity (η) was determined using an Anton Paar microviscosimeter (AMVn) based on the falling-ball principle.[11] The time, t_1, taken by a ball to fall a given distance in a capillary tube of calibrated diameter filled with the liquid, is detected optically. The viscosity of the ILs is calculated from the values of t_1, the capillary calibration constant, K, and the densities of the ball and of the IL, ρ_b and ρ_{IL}, respectively:

$$\eta = K \times (\rho_{IL} - \rho_b) \times t_1 \qquad (1)$$

Two capillaries of 3.0 mm and 1.8 mm nominal diameters were chosen. They were calibrated using appropriate viscosity standards (Cannon Oil). For each IL at each temperature, 12 measurements were made using at least 2 different tilt angles of the capillary. The statistical analysis of the results yielded an estimated uncertainty of the

viscosity of 2%.

Carbon dioxide solubility measurements

A method based on an isochoric saturation technique was used to measure carbon dioxide solubility in all the ionic liquids between 303 K and 343 K and close to atmospheric pressure. The apparatus and the experimental method have been described in previous publications.[7,12] In this technique, a known amount of gaseous solute is put in contact with a precisely determined quantity of degassed solvent at a constant temperature inside an accurately known volume. When thermodynamic equilibrium is attained at a given temperature, the pressure above the liquid solution is constant and is directly related to the solubility of the gas in the liquid. The total uncertainty associated with Henry's law constants calculated from the present experimental solubilities is estimated to be lower than 3 %.

RESULTS AND DISCUSSION

The experimental values obtained for the density of the ILs, at atmospheric pressure and between 293 and 343 K, are reported in Table 2.

Table 2 Experimental densities (ρ) of the studied ionic liquids as a function of temperature and at atmospheric pressure.

$\frac{T}{K}$	$\frac{\rho}{kg.m^{-3}}$	$\frac{T}{K}$	$\frac{\rho}{kg.m^{-3}}$	$\frac{T}{K}$	$\frac{\rho}{kg.m^{-3}}$	$\frac{T}{K}$	$\frac{\rho}{kg.m^{-3}}$
[C_6mPy][Tf_2N]		[C_3OHPy][Tf_2N]		[C_1COOC_2Py][Tf_2N]		[N_{4111}][NTf_2]	
293.14	1356.1	293.15	1551.0	293.20	1532.2	293.26	1396.5
303.17	1347.1	303.24	1541.7	302.68	1522.5	303.22	1387.6
313.14	1338.4	313.14	1532.1	313.17	1512.1	313.03	1379.0
323.13	1329.7	323.15	1522.9	323.04	1501.9	323.13	1370.0
333.22	1321.0	333.16	1513.8	333.25	1492.2	333.17	1361.3
343.00	1312.9	343.13	1504.7	343.19	1482.1	343.18	1352.8
[N_{1132OH}][Tf_2N]		[$N_{1112OOCCH3}$][Tf_2N]		[C_4mpyr][Tf_2N]			
293.16	1453.6	293.18	1486.9	293.09	1398.0		
303.16	1444.5	303.19	1477.3	302.89	1389.2		
313.11	1435.7	313.21	1468.0	313.59	1379.8		
323.14	1426.8	323.16	1458.8	323.38	1371.3		
333.16	1418.1	333.19	1449.7	332.57	1363.1		
343.14	1408.7	343.21	1440.5	342.84	1354.6		

The values obtained are corrected for the viscosity of the samples as recommended in the

literature.[13] The corrected densities were fitted to equation:

$$\ln(\rho/\mathrm{kg.m^{-3}}) = b + a \times (\mathrm{T/K}) \qquad (2)$$

The parameters *a* and *b*, together with the standard deviation of the fit are given in Table 3.

Table 3 Parameters *a* and *b* of Equation (4), and the average relative standard error (σ) from the experimental data.

ILs	*a*	*b*	*σ*
$[C_6mPy][Tf_2N]$	-0.86662	1609.9	0.02%
$[C_3OHPy][Tf_2N]$	-0.92768	1822.9	0.03%
$[C_1COOC_2Py][Tf_2N]$	-1.00007	1825.3	0.06%
$[N_{4111}][Tf_2N]$	-0.87662	1653.4	0.03%
$[N_{1132}OH][Tf_2N]$	-0.89202	1715.0	0.03%
$[N_{1112}OOCCH_3][Tf_2N]$	-0.92571	1758.1	0.04%
$[C_4mPyr][Tf_2N]$	-0.87416	1654.0	0.02%

The densities of some of the ILs studied herein have been measured by other authors: $[N_{4111}][Tf_2N]$[3,14-16] $[N_{1132OH}][Tf_2N]$[17], and $[C_4mPyr][Tf_2N]$[9,16-19] The present results show relative deviations in general lower than ±1%, as shown in Figure 1 for the example of $[C_4mPyr][Tf_2N]$. These small deviations can be explained by the presence of traces of impurities in the samples used, namely water, that can be difficult to eliminate in certain cases.

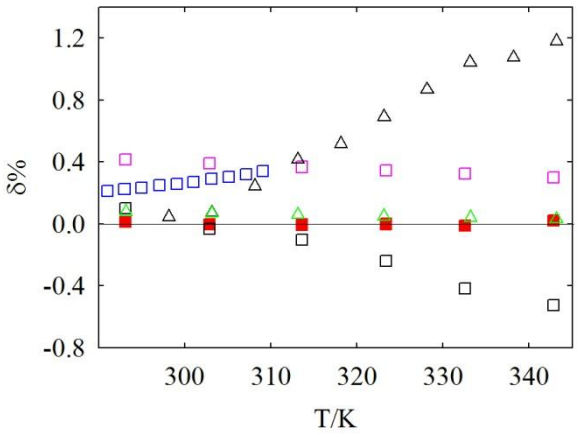


Figure 1. Relative deviations of the density for $[C_4mPyr][Tf_2N]$ from our fitted data: △, Kato *et al.*[18]; △, Hong *et al.*[17]; □, Tokuda *et al.*[16]; □, Anthony *et al.*[9]; □, Kumełan *et al.*[19]; ■, this work.

Group-contribution models have been developed for the prediction of the volumetric properties of ILs as a function of temperature[20] and pressure[21]. These models are based on the assumption that the molar volume of an ionic liquid can be calculated as the sum of the effective molar volumes of the component ions at a given temperature[22]. Using this tool, we have calculated the densities of the functionalized ionic liquids studied in the present work. The effective molar volume of the cations and anions are calculated, according to Jacquemin *et al.*[20], as a sum of contributions:

$$V_{\text{group}}(\text{T}) = \sum_{i=0}^{2} C_i (T - 298)^i \qquad (3)$$

the parameters C_i for different groups being reported in literature[7,20]. Using this prediction scheme, it is possible to predict the molar volumes of the ILs studied herein, as a function of temperature, with average absolute deviations of 1.5%. The value is higher than the uncertainty of 0.5% claimed by Jacquemin *et al.*[20] due to the still inaccurate contribution for the ammonium-based cations calculated using only the few published experimental data[20].

The dynamic viscosities as a function of temperature from 293 to 343 K are listed in Table 4 and are presented in Figure 2.

Table 4 Experimental viscosities (η) in mPa.s of the ILs studied in this work as a function of temperature between 293 K to 343 K at atmospheric pressure.

ILs	293.15 K	303.15 K	313.15 K	323.15 K	333.15 K	343.15 K
$[C_6mPy][Tf_2N]$	105	64.2	41.2	28.3	22.5	17.5
$[C_3OHPy][Tf_2N]$	160	92.2	57.7	38.2	29.4	22.8
$[C_1COOC_2Py][Tf_2N]$	786	338	167	93.0	56.8	37.6
$[N_{4111}][Tf_2N]$	138	82.3	52.2	35.5	27.9	21.5
$[N_{1132OH}][Tf_2N]$	160	92.7	58.1	38.7	30.3	22.9
$[N_{1112OOCCH3}][Tf_2N]$	309	162	90.7	56.8	38.5	27.5
$[C_4mPyr][Tf_2N]$	101	63.8	41.8	28.9	23.5	18.6

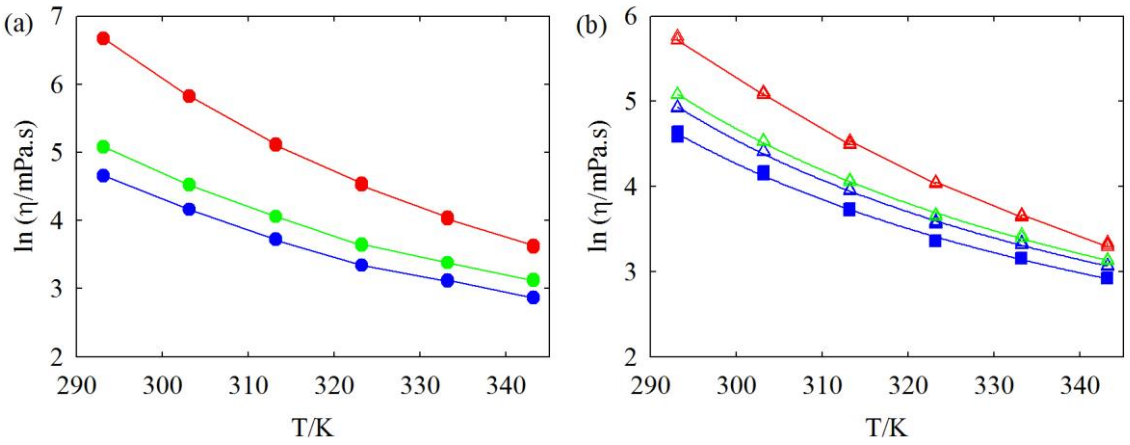


Figure 2. Viscosities as a function of temperature: (a) pyridinium-based ILs: ●, [C_6mPy][Tf_2N] ; ●, [C_3OHPy][Tf_2N]; ●, [C_1COOC_2Py][Tf_2N]. (b) non-aromatic ILs: △, [N_{4111}][Tf_2N]; △, [N_{1132OH}][Tf_2N]; △, [$N_{1112OOCCH3}$][Tf_2N]; ■, [C_4mPyr][Tf_2N]. The lines represent the Vogel-Fulcher-Tamman fit.

The Vogel-Fulcher-Tamman (*VFT*) was used to correlate the experimental viscosities as a function of temperature of ILs[23]:

$$\eta = \eta_0 T^{-1/2} \exp[(B/(T-T_0)] \quad (4)$$

where η_0 is a pre-exponential constant proportional to the viscosity coefficient, and it is assumed as the ideal viscosity at highest temperature. *B* is the pseudo-activation energy for viscous behavior, and T_0 is the ideal glass transition temperature. The values for fitting parameters are given in Table 5. Most of ILs obey the *VTF* law ($\sigma \leq 2\%$)[23]. The ILs with ester group possess higher T_0 values, followed by ILs with hydroxyl group and ILs without oxygen group. There is not evident tendance for the other two parameters.

Table 5 Correlation parameters of the VFT equation (η_0, *B* and T_0) with the standard deviation of the fit (σ).

	η_0/ mPa s	*B*/K	T_0 /K	σ
[C_6mPy][Tf_2N]	26.2	309	220	2%
[C_3OHPy][Tf_2N]	29.7	318	223	2%
[C_1COOC_2Py][Tf_2N]	3.94	709	206	1%
[N_{4111}][Tf_2N]	32.0	303	223	2%
[N_{1132OH}][Tf_2N]	28.2	332	221	2%
[$N_{1112OOCCH3}$][Tf_2N]	6.47	622	200	2%
[C_4mPyr][Tf_2N]	34.8	286	219	2%

At 293.15 K, the lowest temperature measured in this study, the viscosities range from 97 to 786 mPa.s, and at 343.15 K, they are much closer, ranging from 13 to 18 mPa.s. Other research groups have studied the viscosities of some of the ILs and for the alkylmethylpyridinium-based ionic liquids, $[C_4mPyr][Tf_2N]$[23,24] and $[C_6mPy][Tf_2N]$[25], the deviations encountered are of the order of 5%. For $[N_{1112OOCCH3}][Tf_2N]$, the viscosity reported in the literature[23] is up to 41% lower than the present data at 293.15 K, this deviation becoming smaller at the higher temperatures. This high discrepancy is difficult to explain, as the deviations found for other ionic liquids measured by the same authors are much smaller.

The viscosities of the ILs at 303 K are compared in Figure 3 and, as it can be observed, they increase in the order: $[C_4mPyr][Tf_2N]$ < $[C_6mPy][Tf_2N]$ < $[N_{4111}][Tf_2N]$ < $[C_3OHPy][Tf_2N]$ < $[N_{1132OH}][Tf_2N]$ < $[N_{1112OOCCH3}][Tf_2N]$ < $[C_1COOC_2Py][Tf_2N]$. The introduction of oxygen functionalities in the ILs based on the pyridinium or ammonium cations, increases the viscosity, the ester functions having a larger effect that the ether functionalities. The same conclusions have been drawn by Branco *et al.*[26] who have determined the viscosity of ILs with an hydroxyl group on the side chain of imidazolium cations associated to either PF_6^- or BF_4^- anions; by Crosthwaite *et al.*,[25] that studied the viscosity of pyridinium-based ILs containing ester functionalities on the alkyl side chain of the cation; and by Okoturo and VanderNoot[23] who concluded that the ILs with higher molar mass cations containing ester or hydroxyl functional group present higher viscosities. The increase of the viscosity of ILs containing oxygen functionalities (hydroxyl and ester) has been attributed to the additional hydrogen bonding involving these functional groups. The presence of the ether function, in either the cation or the anion of the IL, is considered as a way to decrease the viscosity because the flexibility of the alkyl chains increases.[27] For example, the viscosity of [N-methoxymethyl-N-methylpyrrolidinium][Tf_2N] (41 mPa.s at 298.15K[28]) is higher than that of [N-Methyl-N-propylpiperidinium][Tf_2N] (60 mPa.s[29] or 63 mPa.s[24] at 298.15K). No ILs containing ether functions were studied in this work.

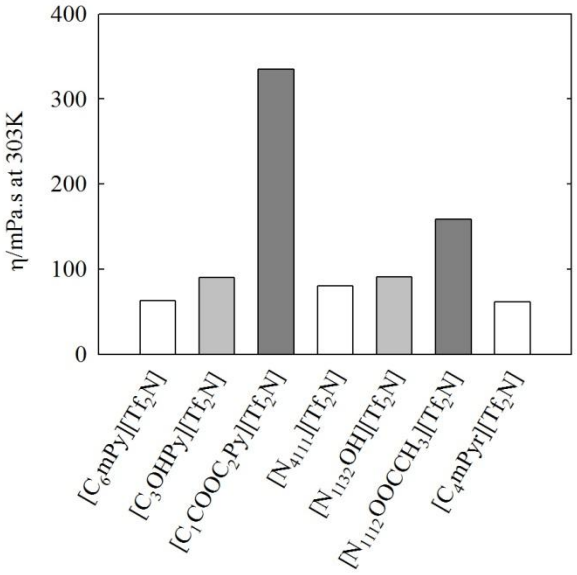


Figure 3. Experimental viscosities of the ILs studied at 303 K

Multiple experimental data points of CO_2 solubilities were obtained for $[C_3OHPy][Tf_2N]$, $[C_1COOC_2Py][Tf_2N]$, $[N_{4111}][Tf_2N]$ and $[N_{1112OOCCH3}][Tf_2N]$ in the temperature interval between 303 K and 343 K in steps of approximately 10 K and close to atmospheric pressure. The experimental solubilities in the different ionic liquids are reported in Table 6.

The experimental solubilities served to calculate the Henry's law constants (K_H), as well as mole fractions of solute (x_2) calculated assuming a partial pressure of the gaseous solute equal to 0.1 MPa. We have taken the values of the second virial coefficients to calculate the compressibility factor and of the fugacity coefficient of the carbon dioxide from the compilation by Dymond and Smith[30].

To correlate the solubility as a function of temperature, the Henry's law constants obtained from the experimental data were adjusted to a power series in 1/T:

$$\ln(K_H / 10^5 Pa) = \sum_{i=0}^{n} A_i (T/K)^{-i} \qquad (5)$$

The coefficients A_i and the average absolute deviations obtained are collected in Table 7. The average absolute deviations characterize the precision of the data, which is of the order of 1 %. In Figure 4 are represented the solubility data expressed in mole fraction as a

function of temperature. The solubility decreases with increasing temperature in the range covered by this work. The solvation takes place preferentially at low temperature, which corresponds to an exothermic process.

Table 6. Experimental values of carbon dioxide solubility in ILs expressed as both Henry's law constants K_H and as mole fraction x_2 corrected for a partial pressure of solute of 0.1 MPa.

T /K	p /10^2 Pa	K_H /10^5 Pa	x_2/10^{-3}	dev	T /K	p /10^2 Pa	K_H /10^2 Pa	x_2/10^{-3}	dev
$[C_3OHPy][Tf_2N]$					$[N_{4111}][Tf_2N]$				
303.58	643.2	84.71	11.75	+ 0.8%	303.55	637.5	36.21	27.49	+ 0.2%
303.59	597.0	84.97	11.71	+ 0.5%	303.57	642.1	36.47	27.29	– 0.5%
313.55	622.5	115.1	8.649	– 0.6%	303.59	666.0	36.3	27.41	+ 0.0%
313.55	616.1	116.3	8.559	– 1.7%	313.53	669.2	43.89	22.69	– 0.3%
323.47	699.6	172.2	5.784	– 2.5%	313.54	434.1	43.24	23.03	+ 1.2%
323.50	648.0	166.9	5.969	+ 0.8%	323.47	693.7	52.00	19.16	– 0.3%
333.42	726.3	264.0	3.775	+ 0.7%	323.52	695.4	52.11	19.12	– 0.4%
333.45	672.7	258.8	3.851	+ 2.9%	333.43	745.6	60.79	16.39	– 0.3%
333.46	665.7	262.4	3.797	+ 1.5%	333.48	720.7	60.33	16.52	+ 0.5%
343.40	752.3	459.0	2.172	– 2.4%	343.41	770.5	69.83	14.28	+ 0.1%
343.45	689.4	449.3	2.219	– 0.2%	343.48	745.5	70.05	14.23	– 0.1%
$[C_1COOC_2Py][Tf_2N]$					$[N_{1112OOCCH3}][Tf_2N]$				
303.59	668.3	73.61	13.52	+ 0.7%	303.58	598.0	37.73	26.38	+ 0.3%
303.60	666.6	73.38	13.56	+ 1.1%	303.68	635.2	38.11	26.11	– 0.5%
313.54	695.5	101.6	9.801	– 2.0%	313.43	661.1	45.32	21.97	– 0.6%
313.55	693.3	101.2	9.842	– 1.6%	313.45	626.0	44.73	22.26	+ 0.8%
323.48	721.9	144.8	6.880	– 1.1%	323.44	687.0	53.65	18.57	– 0.8%
323.50	719.2	142.5	6.991	+ 0.6%	323.46	653.4	52.57	18.95	+ 1.2%
333.44	747.6	215.1	4.634	+ 1.3%	333.39	712.4	63.47	15.70	– 2.1%
333.48	744.6	210.7	4.730	+ 3.6%	333.39	679.8	61.24	16.27	+ 1.4%
343.44	773.0	355.2	2.807	– 2.2%	343.29	736.3	72.47	13.76	– 0.9%
343.50	769.7	349.6	2.851	– 0.3%	343.35	705.6	70.94	14.05	+ 1.3%

Table 7. Parameters of eqn (10) used to smooth the raw experimental results from Table 6 along with the per cent average absolute deviation of the fit (AAD).

IL	A_0	A_1	A_2	AAD %
$[C_3OHPy][Tf_2N]$	72.30	-3.90×10^4	5.58×10^6	1.3
$[C_1COOC_2Py][Tf_2N]$	59.93	-3.14×10^4	4.41×10^6	1.5
$[N_{4111}][Tf_2N]$	6.936	-2.23×10^2	-2.41×10^5	0.4
$[N_{1112OOCCH3}][Tf_2N]$	9.017	-1.58×10^3	-1.55×10^4	1.0

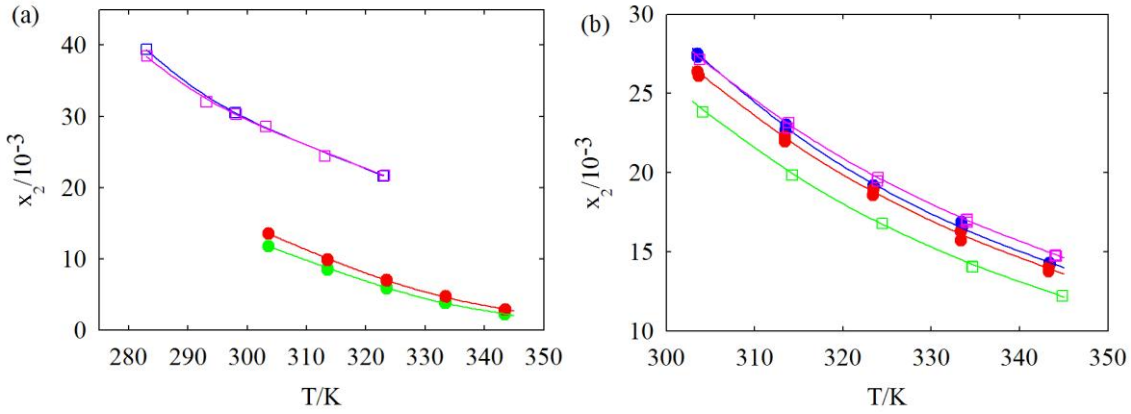


Figure 4. Mole fraction solubility of carbon dioxide in ILs as a function of temperature: (a) pyridinium-based ILs: □, $[C_4mPy][Tf_2N]$[31]; □, $[C_6mPy][Tf_2N]$[32] ; ●, $[C_3OHPy][Tf_2N]$; ●, $[C_1COOC_2Py][Tf_2N]$. (b) non-aromatic ILs: ●, $[N_{4111}][Tf_2N]$; □, $[N_{1132OH}][Tf_2N]$[17]; ●, $[N_{1112OOCCH3}][Tf_2N]$; □, $[C_4mPyr][Tf_2N]$[17].

The CO_2 solubility in $[N_{4111}][Tf_2N]$ has been previously measured by Jacquemin *et al.*[14] using the same experimental technique. The results are is in good agreement with the present data, as observed in Figure 5, with deviations lower than ± 1%. The value reported by Kilaru *et al.*[15] for the CO_2 solubility in the same IL at 303 K only is 40% lower than those reported in this work.

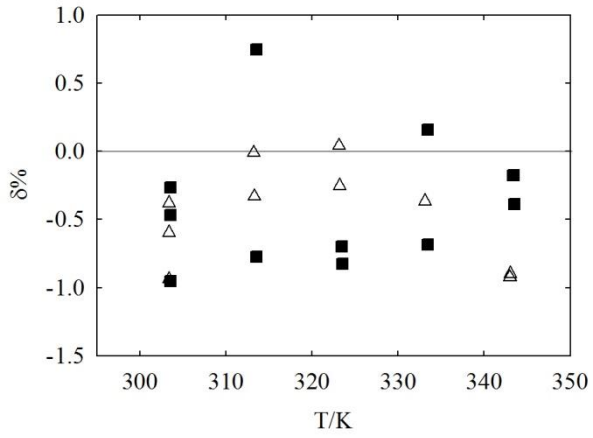


Figure 5. Deviations of the literature values to the correlation of mole fraction solubilities obtained in this work for carbon dioxide in $[N_{4111}][Tf_2N]$: ■, this work; △, Jacquemin *et al.*[14]

Also represented in Figure 4 are the solubilities of CO_2 in other previously studied ILs:

$[C_4mPy][Tf_2N]$[31], $[C_6mPy][Tf_2N]$[32], $[N_{1132OH}][Tf_2N]$[17] and $[C_4mPyr][Tf_2N]$[17]. The CO_2 mole fraction solubilities for these ionic liquids are listed, at five discrete temperatures between 303 and 343 K, in Table 8. These solubilities are compared in Figure 6, at 325 K, with the ones reported herein. All ILs are able to dissolve CO_2 with mole fractions of the order of 10^{-2} at temperatures from room temperature up to 343 K and at partial pressures of gas of the order of 0.1 MPa. Both the imidazolium, the pyridinium, the ammonium and the pyrrolidinium-based ILs are capable of dissolving similar quantities of gas and, except for the pyridinium-based ILs, the presence of hydroxyl or ester functions do not affect significantly the CO_2 mole fraction solubility. In the case of the pyridinium-based ionic liquids, the presence of –OH or –COO– groups, significantly lowers the capacity of the IL to dissolve CO_2.

Table 8. CO2 solubilities in ILs reported in the literature.

T /K	$[C_4mPy][Tf_2N]$[31]		$[C_6mPy][Tf_2N]$[32]		$N_{1132OH}][Tf_2N]$[17]		$[C_4mPyr][Tf_2N]$[17]	
	K_H /bar	$x_2/10^{-3}$	K_H /bar	$x_2/10^{-3}$	K_H /bar	$x_2/10^{-3}$	K_H /bar	$x_2/10^{-3}$
303	35.48	28.18	35.39	28.25	40.78	24.52	36.16	27.66
313	40.61	24.63	40.72	24.56	49.04	20.39	42.77	23.38
323	45.96	21.76	46.20	21.65	58.29	17.16	50.05	19.98
333	51.51	19.42	51.76	19.32	68.58	14.58	57.98	17.25
343	57.22	17.48	57.37	17.43	79.92	12.51	66.57	15.02

For example, the CO_2 solubility in $[C_1COOC_2Py][Tf_2N]$ is almost 50% lower than in $[C_6mPy][Tf_2N]$. Muldoon et al.[6] have reported similar solubilities of CO_2 in the ester-functionalized IL $[C_1COOC_4bPy][Tf_2N]$ and in $[C_6mPy][Tf_2N]$ at atmospheric pressure, even if larger solubilites were expected in the former due to the presence of a longer alkyl-side chain in the cation. The differences found between the influence on the CO_2 solubility of the ester groups associated to imidazolium, ammonium or pyridinium cations are difficult to explain. The negligible effect in imidazolium and ammonium-based ionic liquids has been attributed to the dominant effect of the anion $[Tf_2N]$ or to the proximity of the cation core that lowers the electronegativity of the oxygen of the carbonyl

group[6].

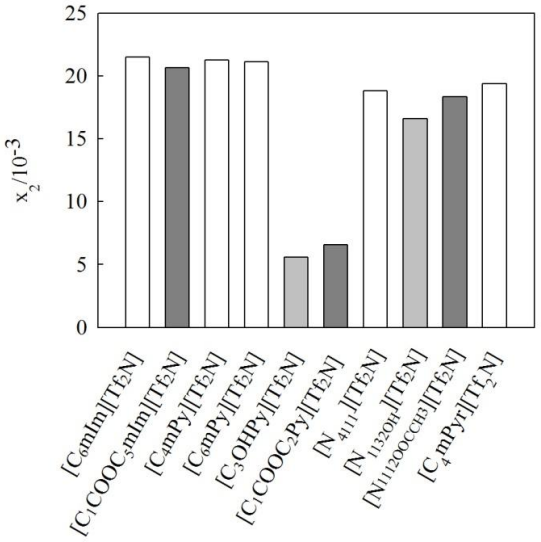


Figure 6. CO_2 mole fraction solubilities in the ILs at 325K corrected for a partial pressure of solute of 0.1 MPa. $[C_6mIm][Tf_2N]$[35]; $[C_4mPy][Tf_2N]$[31]; $[C_6mPy][Tf_2N]$[32]; $[N_{1132OH}][Tf_2N]$[17]; $[C_4mPyr][Tf_2N]$[17].

CO_2 solubility in $[C_3OHPy][Tf_2N]$ is even 15% lower than that measured in $[C_1COOC_2Py][Tf_2N]$. The decrease of CO_2 solubility caused by the presence of a hydroxyl group has also been observed in $[N_{1112OH}][Tf_2N]$[6] and in $[C_2OHmIm][BF_4]$[33]. Free volume arguments as well as weaker interactions between the solute and the IL due to the presence of hydroxyl groups have been advanced as possible explanations for this lowering of CO_2 solubility.[6,34] These explanations do not account for the observations that the presence of hydroxyl groups imidazolium-based ILs with the anions PF_6^- or $CF_3SO_3^-$ cause an increase on the CO_2 solubilities whereas they are comparable in $[C_2OHmIm][Tf_2N]$ and $[C_2mIm][Tf_2N]$.[34]

The thermodynamic properties of solvation provide valuable information about the mechanisms of solvation of the carbon dioxide in the different ILs. The variation of the solubility, expressed in Henry's law constant, with temperature is directly related to the thermodynamic properties of solvation, which, in the case of gaseous solutes at low

pressures, are practically identical to the thermodynamic properties of solution[35]. The Gibbs energy of solvation is given by:

$$\Delta_{sol}G^{\infty} = RT\ln(K_H / p^0) \quad (6)$$

where p^0 is the standard state pressure. The partial molar differences in enthalpy and entropy can be obtained by calculating the corresponding partial derivatives of the Gibbs energy with respect to temperature:

$$\Delta_{sol}H^{\infty} = -T^2\partial / \partial T(\Delta_{sol}G^{\infty} / T) = -RT^2\partial / \partial T\left[\ln(K_H / p^0)\right] \quad (7)$$

$$\Delta_{sol}S^{\infty} = (\Delta_{sol}H^{\infty} - \Delta_{sol}G^{\infty}) / T = -RT\partial / \partial T\left[\ln(K_H / p^0)\right] - R\ln(K_H / p^0) \quad (8)$$

The average values for the Gibbs energy, enthalpy, and entropy of solvation in the temperature range studied are given in Table 9. It can be observed that the larger CO_2 solubility in the ammonium-based ILs cannot be explained by more favourable solute-solvent interactions that would lead to more negative enthalpies of solvation. The more negative enthalpies of solvation are observed in the pyridinium-based ILs, in which the gas is not as soluble as in the ammonium-based ILs. It is a more favorable (less negative) entropy of solvation that explains the larger solubility in the ammonium-based ILs, probably meaning that in these ILs the solute is more mobile, its dissolution leading to a significant increase on the entropy. The oxygen functionalized ILs present, in general, more favorable enthalpies of solvation but less favorable entropic contributions to the Gibbs energy of solvation. The two terms tend to compensate and so the solubility of CO_2 is comparable in fonctionalised and non-fonctionalized ILs. In the case of pyridinium-based ILs, associated with the $[Tf_2N]$ anion, the entropic term is very defavorable leading to significantly lower CO_2 solubilities in hydroxyl or carbonyl functionalized ILs.

Table 9. Average values of the thermodynamic functions of solvation of CO_2 in the four ionic liquids in the temperature range 303 K to 343 K.

	$\Delta_{sol}H^{\infty}$ / kJmol^{-1}	$\Delta_{sol}S^{\infty}$ / Jmol^{-1}K^{-1}
$[C_3OHPy][Tf_2N]$	– 38	– 159
$[C_1COOC_2Py][Tf_2N]$	– 35	– 147
$[N_{4111}][Tf_2N]$	– 14	– 77
$[N_{1112OOCCH3}][Tf_2N]$	– 14	– 76

Conclusion

The presence of carbonyl or hydroxyl groups in the alkyl-side chains of ILs based on the imidazolium, pyridinium, pyrrolidinium or ammonium cations can affect their physico-chemical properties. In previous works[7,8] we have concluded that, for imidazolium-based ILs, the chemical modification of the alkyl side chain does not result in a significant change of the solubility of different families of gases.

Herein, we have studied the incluence of the same chemical modifications in pyridinium and ammonium-based ILs. We conclude that the introduction of oxygen functionalities in the ILs based on the pyridinium or ammonium cations, increases the viscosity, ester functions having a larger effect than the hydroxyl functionalities. The carbon dioxide solubility is not influenced by the presence of the ester and hydroxyl groups in the cation except for the case of pyridinium-based ILs in which the gas is much less soluble when the cation is functionalized. This can be explained by a much less favourable entropic contribution to the Gibbs energy of solvation that largely counterbalances the strong solute-solvent interactions characterised by a relatively large negative enthalpy of solvation.

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Assessing the Environmental Impact of Pyridinium, Ammonium and Pyrrolidinium Based Ionic Liquids

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Abstract Several physico-chemical properties relevant to determine the environmental impact of ionic liquids – aqueous solubility, octanol-water partition coefficient, and infinite dilution diffusion coefficients in water – are studied in this work. The presence of oxygenated functional groups is believed to decrease the environmental impact of ionic liquids by decreasing their toxicity and increasing their biodegradability. We have studied ionic liquids based on pyridinium, ammonium and pyrrolidinium cations and checked the influence of the presence of hydroxyl or ester groups in the physico-chemical properties of these liquids. It was observed that the presence of functional oxygenated moieties reduces the lipophilicity of ionic liquids and so decreases the risk of bioaccumulation in environment.

Keywords: aqueous solubility, octanol-water partition coefficient, infinite dilution diffusion coefficient, ionic liquids, functional group

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1. Introduction

Ionic liquids (ILs) are the ionic compounds, which are liquids at some arbitrary temperature (generally below 100 ℃). They have interesting properties – negligible vapour pressure, thermal stability, high ionic conductivity, electrochemical stability, and tunable solvation properties (Coleman and Gathergood, 2010) – that make them promising candidates as low environemental impact media for different chemical processes. Some prospective work predicts the commercial application of ILs in nearly all of the chemical industry fields (Plechkova and Seddon, 2008) but recent researches have demonstrated that some ILs can be toxic (Pham *et al*., 2010) and resistible to biodegradation (Coleman and Gathergood, 2010). For the development of ionic liquids in wide scale applications, data concerning the environmental impact of this large and diverse family of liquids is necessary. These data includes toxicity, biodegradation, bioaccumulation but also a series of physico-chemical parameters that provide information about the transport and fate of ILs in the compartments of the environment and often serve as a basis for the establishment of reliable prediction models of the environmental impact. (Mackay, 1991; Hemond and Fechner-Levy, 2000).

It is generally considered that the knowledge of the vapour pressure, the aqueous solubility and the partition coefficients of a substance between the different environmental compartments are sufficient to determine its fate in natural ecosystems. The transport of a chemical in the terrestrial compartments, water and soil, can in turn be appropriately modeled from the volumetric properties of the molecule coupled to the knowledge of its aqueous diffusion coefficient. Several specific physico-chemical properties can then be identified as key parameters for the determination of the environmental impact of a chemical substance: the vapour pressure, atmosphere-water partition coefficient, aqueous solubility, octanol-water partition coefficient and aqueous diffusivity (Mackay, 1991). Because ionic liquids have a negligible vapour pressure, no presence of ILs in a gaseous phase is expected at environmental conditions and so this list of properties is reduced to the three last ones.

The aqueous solubilities of ILs are characterized by a highly pronounced anion effect.

Some anions render the ILs fully miscible with water at ambient temperature, *e.g.* Cl^-, NO_3^-, OAc^-, and CF_3COO^- (Schrekker *et al*., 2007; Freire *et al*., 2009). The solubilities of hydrophobic ILs can be fine-tuned by the cations structures. For instance, a longer alkyl chain usually makes the IL less soluble in water (Freire *et al*., 2008; Freire *et al*., 2009). The diffusion coefficient of ILs in water is rarely reported, while only imidazolium ionic liquids with different anions are studied (Su *et al*., 2007; Wong *et al*., 2008; Sarraute *et al*., 2009; Deng *et al*., submitted).

The octanol-water partition coefficient (K_{OW}) is defined as the ratio of the equilibrium concentrations of the test substance in 1-octanol saturated with water (C_O) and in water saturated with 1-octanol (C_W) (OECD 123). It is an important factor determining movements and distributions of chemicals in the environment (Sangster, 1997). It is accepted for a long time as one of the quantitative physical properties that correlates best with biological activity, the soil sorption constant, and parameters predicting biological, biochemical and toxic effects (Danielsson and Zhang, 1996; Ropel *et al*., 2005; Toropov and Raska, 2008). It is widely used as a parameter for quantitative structure–activity relationship (QSAR) and plays an important role in the early stages of an environmental risk assessment for a chemical (Danielsson and Zhang, 1996; Berthod and Carda-Broch, 2004).

Even if the K_{OW} is a key parameter for assessing the environmental impact of a chemical substance, it has been investigated rarely for ILs. Few studies (Ropel *et al*., 2005; Lee and Lee, 2009; Choua *et al*., 2003; Deng *et al*., submitted) have used direct measurement methods to test imidazolium ILs, while some authors have predicted K_{OW} of from their structures (Stepnowski and Storoniak, 2005). Domanska *et al*. (Domanska *et al*., 2003), and Chapeaux *et al.* (Chapeaux *et al*., 2007) have used indirect methods to obtain the K_{OW} of some imidazolium, pyridinium and quaternary ammonium based ILs, from the solubility of ILs in pure water and in pure 1-octanol, a value that does not correspond to the exact definition of K_{OW}. At present, the authors found no published direct measurements of K_{OW} of ILs based on cations other than imidazolium.

The reported K_{OW} values of 3-methyl-1-alkyl-imidazolium ([$C_n$mim]) based ILs have huge discrepancies but still some tendencies can be identified. Except for [C_6mim][Tf_2N]

and [C_8mim][Tf_2N] the imidazolium based ILs have values of K_{OW} lower than 1. For the ILs with same anion, the values of K_{OW} increase as the alkyl side chain on the cation increases. K_{OW} of ILs with [Tf_2N]$^-$ are larger than those of ILs with other anions, eg. [Br]$^-$, [BF_4]$^-$, [NO_3]$^-$, [Cl]$^-$ and [PF_6]$^-$, while those of ILs with [Br]$^-$, [NO_3]$^-$ or [Cl]$^-$ are extremely low.

Some researchers have reported that the introduction of ester or ester plus ether groups, which are susceptible to enzymatic hydrolysis, in the alkyl side chains of imidazolim (Gathergood *et al*., 2004; Garcia *et al*., 2005; Gathergood *et al*., 2006) and pyridinium (Harjani *et al*., 2009) based ionic liquids also can greatly improve their biodegradability. Other properties characterizing the environmental impact of the oxygen functionalized ILs have only been determined for imidazolium based ILs (Deng *et al.*, submitted) and, to our knowledge, only rarely for non-imidazolium based ILs. In the present work, we have studied the aqueous solubility, octanol-water partition coefficient and the aqueous diffusivity of ILs based on pyridinium, pyrrolidinium and ammonium cations and on the bis(trifluoromethylsulfonyl)imide anion ([Tf_2N]$^-$). We have checked the influence of the presence of oxygenated functional groups (hydroxyl or ester) on these properties and so could estimate their effect on the environmental impact of the ILs.

2. Materials and methods

2.1. Materials

The structures and abbreviations of the ILs used in this study are listed in Fig. 1. Three pyridinium based ILs ([C_3Py][Tf_2N], [C_1COOC_2Py][Tf_2N], [C_3OHPy][Tf_2N]), and one pyrrolidinium based IL ([C_1mPyr][Tf_2N]) were purchased from Iolitec (Germany) and their purity is claimed to superior to 98%. One ammonium based IL ([$N_{1112OOCCH3}$][Tf_2N]) was purchased from Solvionic (France) and its purity is claimed to superior to 98%. The other ILs ([C_6mPy][Tf_2N], [C_4mPyr][Tf_2N], [N_{4111}][Tf_2N] and [N_{1132OH}][Tf_2N]) were supplied by Queen's University of Belfast, UK. Their purity is above 99% ^{1}H NMR. The water used in this study was distilled water.

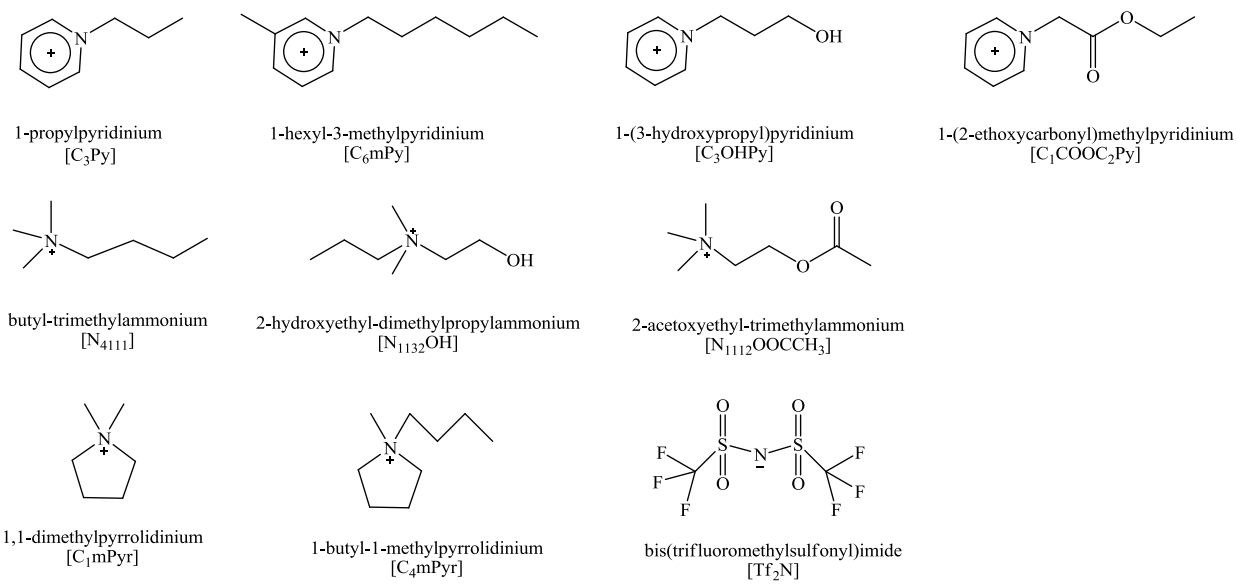

Fig. 1. Abbreviation, structures and names of ions in the studied ILs

2.2 Aqueous solubility measurements

The solubility of the different ILs in water was measured at 22 ℃ and at ambient pressure following the procedure described in the *OECD Guideline 105* (OECD 105). A two-phase mixture of IL and distilled water was slowly stirred for 48 hours at 25 ℃, and then was left at 22 ℃ for 24 hours. The concentration of IL in the liquid phase was measured using an UV-vis spectrometer (*UVIKON 941*). The total uncertainty of the measurements was evaluated as 4%.

In the case of the pyrrolidinium based and the ammonium based ILs, their aqueous solubility was determined visually. A two-phase mixture (water-IL) was prepared and then water was added until the disappearance of the IL phase after stirring for 24 h.

2.3 Octanol-water partition coefficient measurement

The K_{OW} of pyridinium based ILs were measured following the *OECD Guideline 123* (OECD 123) at room temperature (22 ± 1 ℃). The vial containing the system of water pre-saturated with 1-octanol, 1-octanol pre-saturated with water, and IL was closed tightly and stirred slowly using a Teflon coated stir bar for at least 48 hours (the vortex depth

never exceeded 1 cm). Concentrations of ILs in each phase were measured by UV-vis spectroscopy (*UVIKON 941*). The detailed procedure is described in a previous work (Deng *et al*., submitted).

The K_{OW} of pyrrolidinium and ammonium based ILs were not measured because these coumpounds could not be detected using the UV-vis spectrophotometer.

2. 4 Diffusion coefficient Measurements

Diffusion coefficients at different temperatures were measured using the Taylor dispersion technique, whose details were reported previously (Sarraute *et al*., 2009; Deng *et al*., submitted). The overall scatter of the data points corresponds to a precision of 0.02 $\times 10^{-9}$ $m^2.s^{-1}$, corresponding to a (1 - 2)% relative error associated with the data.

3. Results and Discussion

All ILs studied are only partially miscible with water. The solubilities of the ILs in water measured in this work and reported in the literature at 22 ± 1 ℃ are listed in Table 1. The values range from 3.55–175 $mmol.L^{-1}$ or (0.65–32) $\times 10^{-4}$ in mole fraction.

A ^1H NMR analysis shows that, after three days of contact with water, 9% $[C_1COOC_2Py][Tf_2N]$ hydrolyses following the reaction in Fig. 2. The hydrolysis product and the IL are simultaneously measured by UV spectrometry (they absorb at the same wavelength) and as a consequence it is not possible to determine the concentration of pure IL in the solution. So the value indicated in Table 1 as being the solubility of $[C_1COOC_2Py][Tf_2N]$ represents the total concentration of the two pyrrolidinium cations.

Table 1. Experimental solubility of ILs in water expressed in milimole per litre (*s*/ mmol.L^{-1}) and in mole fraction (x_{IL}) at 22 ±1 ℃.

ILs	*s*/ mmol.L^{-1}	$10^4 x_{IL}$	Reference/Meas. Method
$[C_3mPy][Tf_2N]$	20.8 ± 0.1	3.75 ± 0.02	Freire *et al*., 2008 [a], UV
$[C_3Py][Tf_2N]$	32 ± 2	5.7 ± 0.4	this work, UV
$[C_6mPy][Tf_2N]$	3.55 ± 0.08	0.64 ± 0.01	this work, UV
	3.7 ± 0.2	0.67 ± 0.04	Chapeaux *et al*., 2007[b], UV
$[C_3OHPy][Tf_2N]$	175 ± 7	32 ± 1	this work, UV
	207	37	Ranke *et al*., 2009[c], HPLC-UV
$[C_1COOC_2Py][Tf_2N]$	35 ± 2	6.6 ± 0.4	this work, UV
$[N_{4111}][Tf_2N]$	29 ± 4	4.5 ± 0.6	this work, visual
$[N_{1132OH}][Tf_2N]$	104 ± 5	19 ± 1	this work, visual
$[N_{1112OOCCH3}][Tf_2N]$	63 ± 1	12 ± 1	this work, visual
$[C_1mPyr][Tf_2N]$	45 ± 2	8.4 ± 0.4	this work, visual
$[C_4mPyr][Tf_2N]$	14 ± 1	2.7 ± 0.2	this work, visual
	15	2.69	Ranke *et al*., 2009 [c], HPLC-ESI-MS
	14 ± 1	2.5 ± 0.2	Alfassi *et al*., 2003, ESI-MS
	14-15	2.5-2.7	Salminen *et al*., 2007 [a], visual

[a] at 25 ℃, [b] at 23.5 ± 1 ℃, [c] at 20 ℃

Fig. 2. Reaction of hydrolysis of $[C_1COOC_2Py][Tf_2N]$ in water.

The aqueous solubility of $[C_4mPyr][Tf_2N]$ determined by different methods (Alfassi *et al*., 2003; Salminen *et al*., 2007; Ranke *et al*., 2009) shows a good reproducibility, the values laying to within 3%. The result for $[C_6mPy][Tf_2N]$ is also in good agreement with

the work of Chapeaux *et al.* (Chapeaux *et al*., 2007), the measurements for $[C_3OHPy][Tf_2N]$ showing a 10% deviation. (Ranke *et al*., 2009)

For alkyl side-chains shorter than 6 carbon atoms, the aqueous solubilities of the different ILs follow the order: $[C_n mim] > [C_n mpyr] > [C_n mpy]$. When the number of carbon atoms reaches 8, the solubilities of all the IL based on all the three kinds of cations are almost the same, between 1 and 3 $mmol.L^{-1}$. The substituent methyl on 3-position of pyridinium ring reduces the ILs solubility in water (Table 1).

The hydroxyl and ester groups in the side chain of the cation always increase the solubilities of ILs in water. Herein, we have observed that the hydroxyl group increases by one order of magnitude the aqueous solubility for both pyridinium and ammonium based ILs, in agreement with the behavior of imidazolium based ILs (Chapeaux *et al*., 2007). These high solubilities are probably caused by the forming hydrogen bond between solute and solvent.

The experimental aqueous diffusivity ILs at infinite dilution (D_{AB}) are listed in Table 2. The total uncertainty of the diffusion coefficients was evaluated as $\pm 5\%$ by error propagation analysis taking into account the various parameters of the experimental method.

The experimental data were fitted to Arrhenius plots of the form:

$$\ln(D_{AB}) = \ln D_0 + \frac{E_a}{RT} \qquad (1)$$

where D_0 represents the diffusion coefficient at infinite temperature, and E_a the activation energy of the solute for diffusion. The values for E_a and D_0 along with the relative standard deviation are also listed in Table 2. The values for D_{AB} vary from 0.65 to 0.82 $\times 10^{-9}$ $m^2.s^{-1}$ at 20 ℃ (Table 2). Because the solutions were always prepared just before each measurement, the hydrolysis of $[C_1COOC_2Py][Tf_2N]$ could be ignored. The coefficients increase with temperature almost linearly in the temperature range considered (10 ℃ to 30 ℃), following the Arrhenius plots, the same behavior was already observed with the imidazolium-based ILs, both non-functionalized (Sarraute *et al*., 2009) and functionalized (Deng *et al*., submitted).

Table 2. Experimental values of diffusion coefficients for ILs in water at infinite dilution and parameter for fitting eq. (1) from 10 ℃ to 30 ℃. σ is the standard deviation of the fit.

ILs	$D_{AB}/10^{-9}m^2.s^{-1}$			E_a	D_0	σ
	10 ℃	20 ℃	30 ℃	kJ.mol^{-1}	$10^{-11}m^2.s^{-1}$	
$[C_3Py][Tf_2N]$	0.56	0.76	1.0	-20.7	36.9	0.2%
$[C_6mPy][Tf_2N]$	0.49	0.65	0.87	-20.5	29.2	0.6%
$[C_3OHPy][Tf_2N]$	0.56	0.77	0.98	-20.0	27.6	1.3%
$[C_1COOC_2Py][Tf_2N]$	0.51	0.71	0.93	-21.5	46.6	0.9%
$[N_{4111}][Tf_2N]$	0.55	0.79	1.1	-23.8	134	0.8%
$[N_{1132OH}][Tf_2N]$	0.53	0.72	0.92	-19.7	23.0	0.9%
$[N_{1112OOCCH3}][Tf_2N]$	0.55	0.73	0.97	-20.2	29.7	0.5%
$[C_1mPyr][Tf_2N]$	0.60	0.82	1.1	-20.0	29.4	1.0%
$[C_4mPyr][Tf_2N]$	0.53	0.72	0.93	-20.1	26.9	0.7%

In Fig. 3, the diffusion coefficients of these ILs in water are compared with those of other families of chemical compounds. It is observed that they are similar with those of N-methylpyrrolidone (Riele *et al*., 1995), and those of different alcohols (Riele *et al*., 1995; Funazukuri and Nishio, 1999; Tominaga and Matsumoto, 1990), and alpha-amino acids (Umecky *et al*., 2006) with molecular weight (*M*) varying from 90 to 200; lower than those with *M* lower than 80; but higher than the poly(ethylene glycol) and poly(propylene glycol) with similar *M* 200-600 (Chin *et al*., 1991). These observations prove that the diffusivities of ILs in water depend, in the present case, not only of the molecular weight of these compounds but also on the specific solute-solvent interactions.

The differences in diffusivity observed between the ILs containing same anion $[Tf_2N]$ but different cation families is not easily explained. For the ILs with the same cation core, the diffusion coefficient decreases with the increase on the length of the side chain on the cation. The presence of hydroxyl groups (light gray bars in Fig. 3) and ether group (dark gray bars) seems to reduce the diffusivity slightly, which might be simply attributed to the increase on the length of the side-chain length.

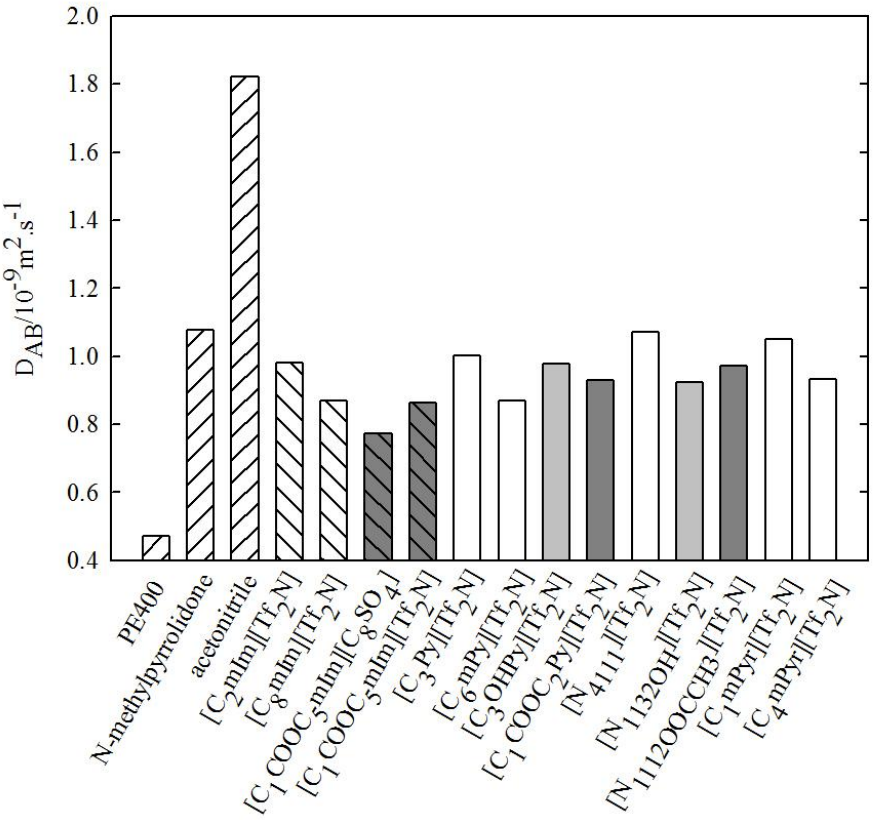

Fig. 3. D_{AB} of compounds at 30 ℃: organic compounds (Chin *et al.*, 1991); non-functionalized imidazolium based ILs (Sarraute *et al.*, 2009) and functionalized ones (Deng *et al.*, submitted. $[C_1COOC_5mim]$ = 3-methyl-1-(pentoxycarbonylmethyl)imidazolium, $[C_1COOC_2OC_2OC_4mim]$ = 3-methyl-1-(2-(2-butoxyethoxy)ethoxycarbonylmethyl) imidazolium).

The diffusion coefficient of an IL infinitely diluted in water is normally proportional to its molecular weight. In order to check if this dependence is respected for the ILs studied in this work, the diffusion coefficients measured are plotted in Fig. 4 (a) as a function of their molecular weight. The imidazolium based ILs with Tf_2N^- (Sarraute *et al.*, 2009), BF_4^- (Su *et al.*, 2007), PF_6^- (Su *et al.*, 2007) and some other anions (Wong *et al.*, 2008) reported in literature are also plotted. The 32 experimental points are adjusted to a straight line ($D_{AB} \times 10^{-9} = -0.0015 \times M + 1.5973$, with DAB in $m^2.s^{-1}$) with a rather poor coefficient of determination ($R^2 = 0.72$). This empirical correlation can predict the diffusion coefficient at 303 K for most of the Tf_2N^- anion based ILs with standard error less than 5%.

For some of the ILs with other anions, the predicted values are rather far away from the experimental data, suggesting that in this model, the contribution of cation and anion should be considered separately and surely more experimental data are needed to increase the precision of the parameters. Furthermore, this model does not take into account the temperature dependence of the diffusivity of the ILs in water.

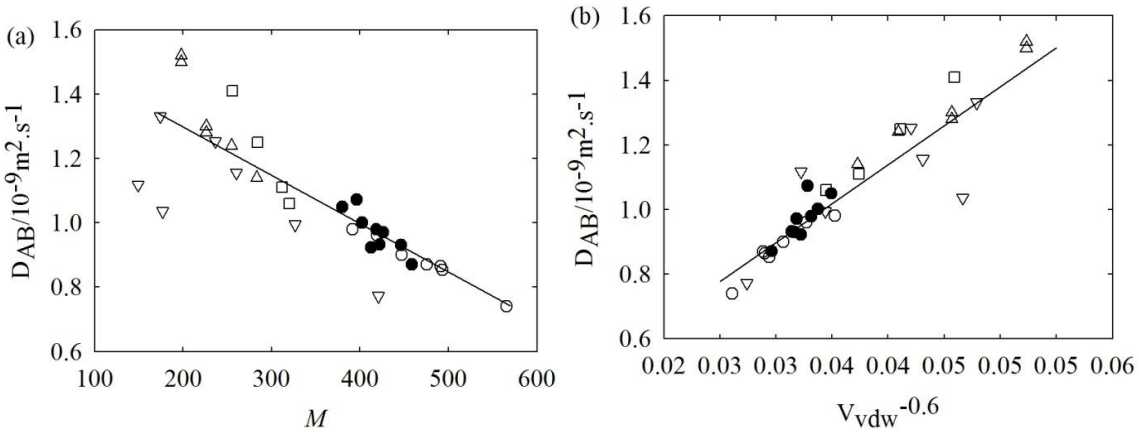

Fig. 4 (a) Molar mass and (b) V_{vdW} dependences of diffusion coefficient for ILs at 30 ℃: ●, ILs containing [Tf_2N^-] (Sarraute *et al*., 2005, Deng *et al*., submitted); △, ILs containing [BF_4^-] (Su *et al*., 2007, Sarraute *et al*., 2005); □, ILs containing [PF_6^-] (Su *et al*., 2007), ∇, ILs containing other anions (Wang *et al*., 2008, Sarraute *et al*., 2005).

Wilke–Chang equation is commonly used for estimating diffusion coefficient of organic molecules (A) in a solvent (B) (Su *et al*., 2007; Wong *et al*., 2008; Sarraute *et al*., 2009):

$$D_{AB}(T) = 7.4 \times 10^{-12} \frac{(\psi M_B)^{0.5} T}{\eta_B V_A^{0.6}} \qquad (2)$$

where ψ is the association parameter for the solvent (we have consider it equal to 2.26 for water as recommended by Wong *et al*. (2008)). M_B and η_B are the molar mass and viscosity of solvent (in g.mol^{-1} and in cP, respectively). V_A is the molar volume of the solute in cm^3.mol^{-1} at its normal boiling point. The viscosity values of water at 10 ℃, 20 ℃, 30 ℃ were considered as 1.307, 1.002 and 0.7975 cP, respectively (Weast, 1970). As shown in other studies on the diffusion of ILs in water (Su *et al*., 2007; Wong *et al*., 2008; Sarraute *et*

al., 2009; Deng *et al*., submitted), Wilke–Chang equation tends to underestimate the diffusion coefficient of imidazolium, pyridinium, ammonium and pyrrolidinium based ILs. A modified Wilke–Chang equation has been proposed in the work of Deng *et al*. (Deng *et al*., submitted) for imidazolium based ILs that was able to give a satisfying predictive result for these ILs with other cations:

$$D_{AB}(T) = 8\times10^{-11}\frac{T}{\eta_B V_{vdw}^{0.6}} \qquad (3)$$

The results obtained by this semi-empirical model are represented in Fig. 4 (b) and it can be observed that some of the diffusivities are not well predicted (deviations of up to 37%), the temperature dependence being nevertheless included in this case. Due to the diversity of possible solute-water interactions and to the lack of experimental data, the predictions of the diffusivity of different ILs in water are still not very precise. At present, different predictive models of diffusion coefficient of ILs in water should used for different ILs families, depending on the molecular nature of the cations and anions.

The octanol-water partition coefficients, K_{OW}, of the pyridinium based ILs were determined at 22 ± 1 ℃. The values measured are reported in Table 3 and are represented in Fig. 5. The K_{OW} for the different ionic liquids are obtained by extrapolating the measured values to zero aqueous concentrations in order to obtain the partition coefficients at infinite dilution (Sangster, 1997). The observed dependence with the concentration of the partition coefficients is the result of the different dissociation of the ILs in the water-rich or the octanol-rich phases. This dependence with concentration has been observed by other research groups (Danielsson and Zhang, 1996; Ropel *et al*., 2005; Domanska *et al*., 2003). The measured values can be considered as apparent octanol-water partition coefficients or distribution coefficients, their relation to K_{OW} being precisely established by the knowledge of the difference between the degree of dissociation of the ILs in the two phases in equilibrium. In light of these considerations, we have then considered the extrapolated values for infinitely diluted aqueous solutions (K_{OW}^{∞}) as the K_{OW} for the studied ILs.

Table 3. Experimental K_{ow} values of pyridinium based ILs at 22 ±1 ℃.

C_w/ mmol.L^{-1}	K_{ow}	C_w/ mmol.L^{-1}	K_{ow}	C_w/ mmol.L^{-1}	K_{ow}	C_w/ mmol.L^{-1}	K_{ow}
[C_3Py][Tf_2N]		[C_6mPy][Tf_2N]		[C_3OHPy][Tf_2N]		[C_1COOC_2Py][Tf_2N]	
K_{OW}^{∞} = 0.04		K_{OW}^{∞} = 1.39		K_{OW}^{∞} = 0.02		K_{OW}^{∞} = 0.03	
0.31	0.05	0.28	2.12	0.76	0.02	0.17	0.02
0.34	0.05	0.29	2.10	1.49	0.03	0.18	0.04
0.42	0.06	0.29	2.17	2.80	0.05	0.20	0.02
0.61	0.06	0.40	2.24	4.32	0.06	0.42	0.05
0.64	0.06	0.41	2.33	7.25	0.07	0.44	0.05
0.65	0.07	0.41	2.29			0.45	0.05
0.91	0.08	0.41	2.49			0.69	0.03
0.97	0.08	0.42	2.49			0.96	0.05
1.02	0.08	0.44	2.39			0.99	0.06
1.19	0.10	0.49	2.61			1.66	0.07
1.24	0.10	0.49	2.99			1.77	0.07
1.31	0.09	0.52	3.04			2.06	0.08
1.46	0.11	0.54	2.88			2.09	0.08
1.51	0.10	0.57	2.61				
1.52	0.10	0.57	2.55				

The analysis of the values for [C_1COOC_2Py][Tf_2N] is more complicated because of the occurrence of a hydrolysis reaction, as described above. Due to the difficulty to analyse the concentration of the individual species, the exact value for K_{OW} is not possible to obtain using the present experimental technique. The reported values are apparent partition coefficients, corresponding to the ratio of the concentrations of all species containing aromatic rings in two phases in equilibrium.

The K_{OW} of [C_6mPy][Tf_2N] was much higher than the other ILs studied herein. This is caused by the longer alkyl side-chain in the cation that increases the hydrophobilicity of the ILs. The introduction of ester group the alkyl chain of the cation reduces K_{OW} significantly, the hydroxyl group having a more moderate effect.

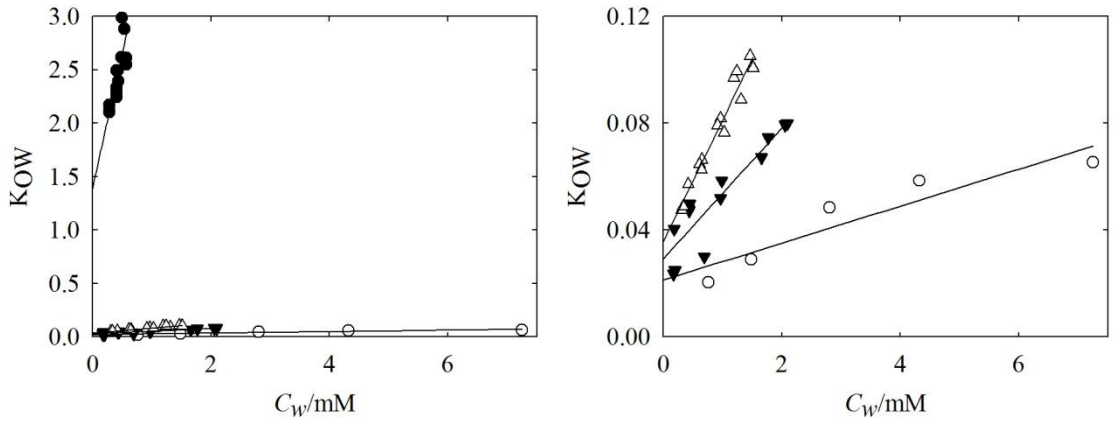

Fig. 5. Experimental K_{OW} values of pyridinium-based ILs at 22 ±1 ℃: Δ, $[C_3Py][Tf_2N]$; ●, $[C_6mPy][Tf_2N]$; ○, $[C_3OHPy][Tf_2N]$; ▼, $[C_1COOC_2Py][Tf_2N]$.

The values of K_{OW} range from 0.02 to 1.39 for the pyridinium based ILs and are compared with other families of ILs and with different common organic solvents in Fig. 6. The K_{OW} of $[C_6mPy][Tf_2N]$ is comparable with that of $[C_8mim][Tf_2N]$ (Ropel *et al*., 2005), and is one order of magnitude higher than $[C_6mim][Tf_2N]$ (Ropel *et al*., 2005). This demonstrates that the pyridinium cation causes the IL to be more lipophilic than those based on the imidazolium cation. Compared to some classical solvents, the K_{OW} values of ILs are similar to those of low molecular weight polar solvents (except halogen solvents), much lower than those of polar solvents and apolar solvents.

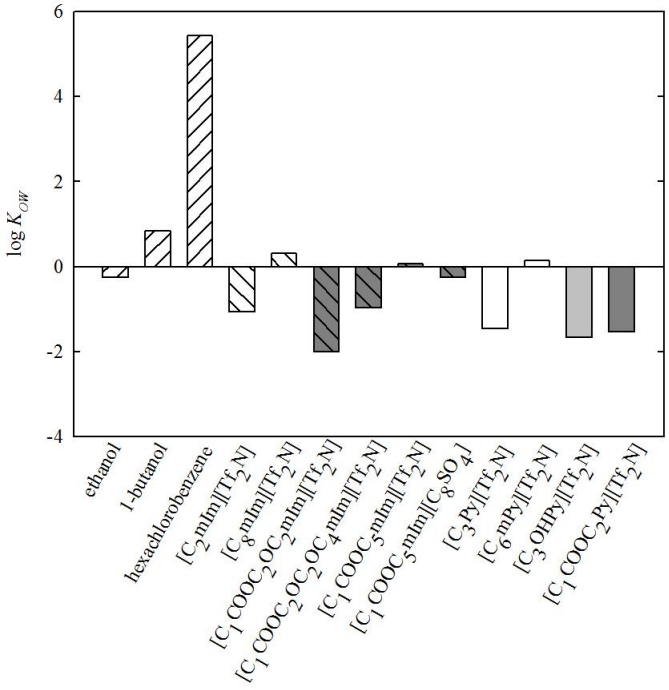

Fig. 6. log K_{OW} values of different compounds at 20-25 ℃: classic solvents (Sangster, 1997); non-functionalised imidazolium IL (Ropel *et al*., 2005); functionalised imidazolium ILs (Deng *et al*., submitted).

Conclusion

In this work, the effect of molecular structures on the physico-chemical properties controlling the environmental impact of chemicals – aqueous solubility, infinite dilution diffusion coefficient in water and partition coefficient octanol-water of ILs are studied. The presence of ester and hydroxyl group influenced inconspicuously the diffusion coefficient, which is simply in inverse proportion to the molecular volume (molecular weight) of the ILs. The ester group introduced in the middle of alkyl chain decreases the lipophilicity caused by the alkyl chain, so the aqueous solubility is increased and the octanol-water partition coefficient is reduced. The presence of hydroxyl groups in the alkyl chains of the cations increase the ILs aqueous solubility to a lower extent but still considerably reduce the octanol-water partition coefficient.

The low K_{OW} values for all the pyridinium based ILs provide important information about impact of these chemicals in the ecosystems. They indicate that these ILs have low

possibility of passive diffusion across a biological membrane, of being stored in fat depots or in other lipophilic sites such as membranes or lipoproteins. If the ILs are absorbed in vertebrates, they will tend to dissolve more (or nearly same) in blood water and will associate less with lipoproteins and membranes of blood cells, and difficult to cross the blood-brain barrier to move into the brain. For aquatic organisms, low biocentration factor are associated with low K_{OW} values. If these ILs enter terrestrial ecosystems, they tend to dissolve in water and are adsorbed to soil colloids only to a limited degree. As consequence, they have low possibility of persistently transferring along terrestrial food chains and low toxic effect towards the organisms.

Acknowledgement

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5. Results and Conclusions

5.1Density

The $[Tf_2N]^-$ based ionic liquids exhibit general higher densities than $[C_8SO_4]^-$ and $[OAc]^-$ based ones. The inclusion of oxygen functional groups seemed slightly increasing the densities of ionic liquids. We have completed the group contribution model to predict the density of ionic liquids of Jacquemin *et al.*[39] by calculation of contributions of ether and ester groups. Most of values are coherent with the uncertainty of 0.5% claimed by Jacquemin *et al.*, except the ionic liquids with the groups rarely studied.

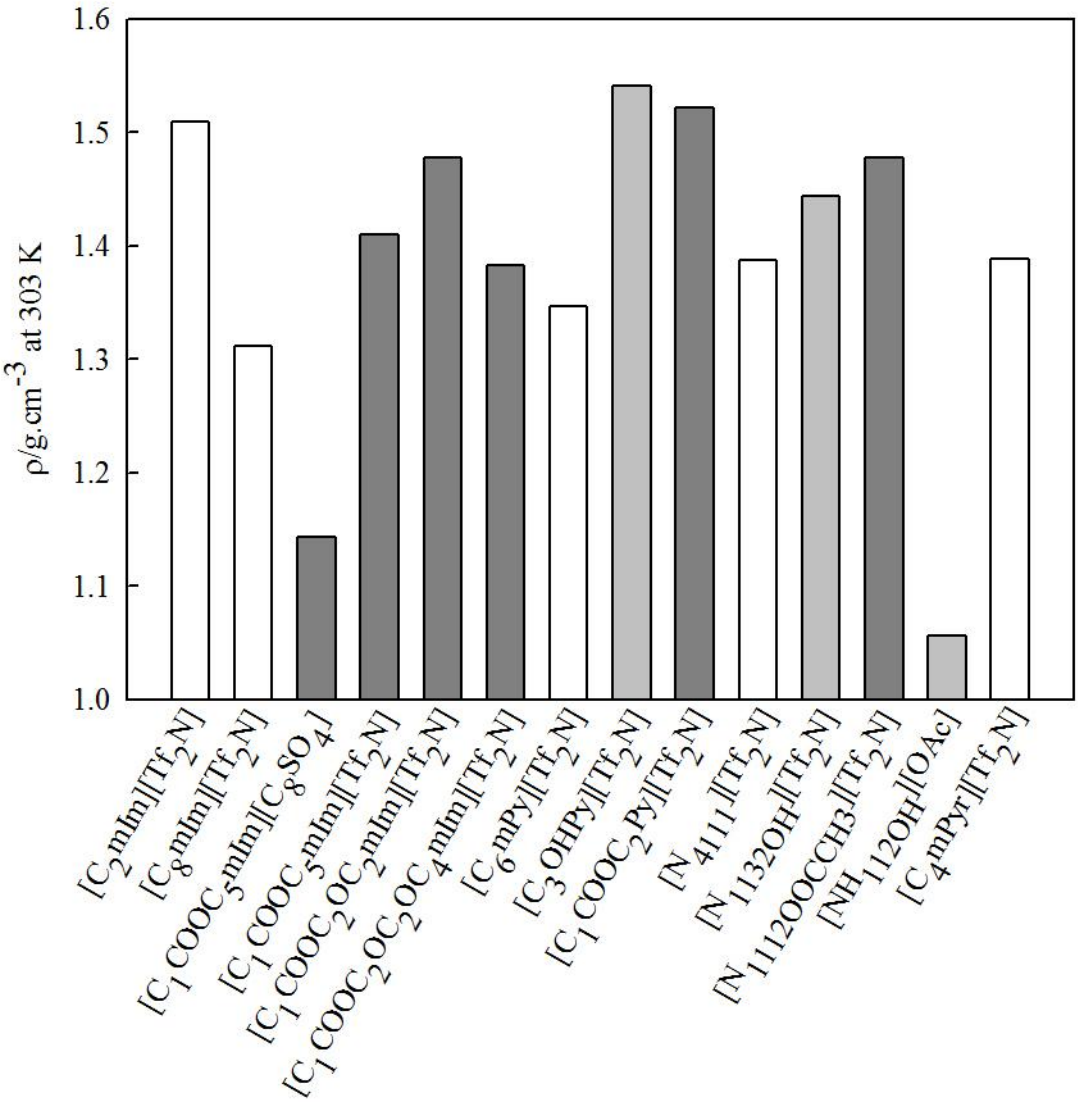

Figure 5.1 Densities of ionic liquids at 303K. The ionic liquids containing a ester group are in dark grey, the ones containing a hydroxyl group in light grey and the non-functionalised ones are in white.

5.2 Solvation properties

A. Gas solubility

In the studied imidazolium-based ionic liquids, carbon dioxide is the most soluble gas, followed by ethane and methane. All of the solubilities decrease with increasing temperature, corresponding to an exothermal solvation process.

The CO_2 solubilities in the ionic liquids with the four different head groups are of the same order of magnitude, except the oxygen-functionalized pyridinium-based ones. It is observed that CO_2 solubility increases with the increase of the alkyl-side chain of the imidazolium cation[51,227], but the rise is less important for longer alkyl chains, especially above eight carbon atoms.[228,229] However, the lengthening of side chains with ether groups can keep the increasing of solubility with chain length.

The introduction of oxygen functional groups in the cations of the imidazolium and ammonium-based ionic liquids did not result in a significant change of the carbon dioxide solubilities. In the case of the pyridinium based ionic liquids, the solubility of carbon dioxide significantly decreases due to a defavourable entropic contribution to the Gibbs energy of solvation.

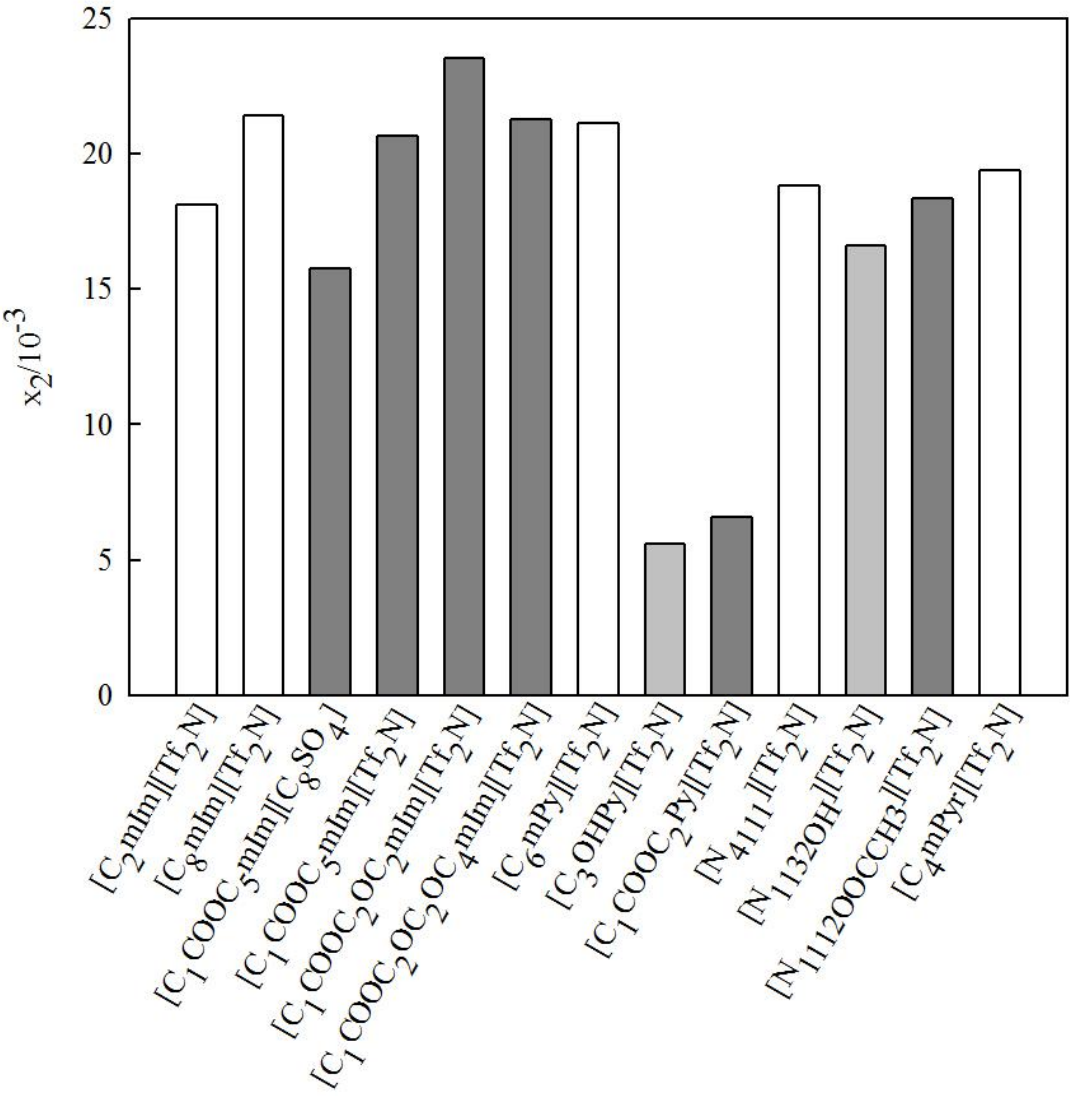

Figure 5.2 CO_2 mole fraction solubilities in the ionic liquids at 325K corrected for a partial pressure of solute of 0.1 MPa. The ionic liquids containing a ester group are in dark grey, the ones containing a hydroxyl group in light grey and the non-functionalised ones are in white.

B. Aqueous solubility

The ionic liquids with $[C_8SO_4]^-$ or $[OAc]^-$ anions are completely miscible with water, whilst the $[Tf_2N]^-$ based salts are partially soluble in water.

For the non-functionalized ionic liquids with the same head group, their aqueous solubilities decreased with the alkyl chain lengths. The solubilities of the three imidazolium salts containing ester group decrease with their molecular weights. Compared to the non-functionalized ionic liquids, the aqueous solubility of ester-functionalized salts are higher but still of the same order of magnitude; while the presence of hydroxyl group enhances the solubility one order of magnitude, probably due to the formation of hydrogen bonds between the solute molecules and water.

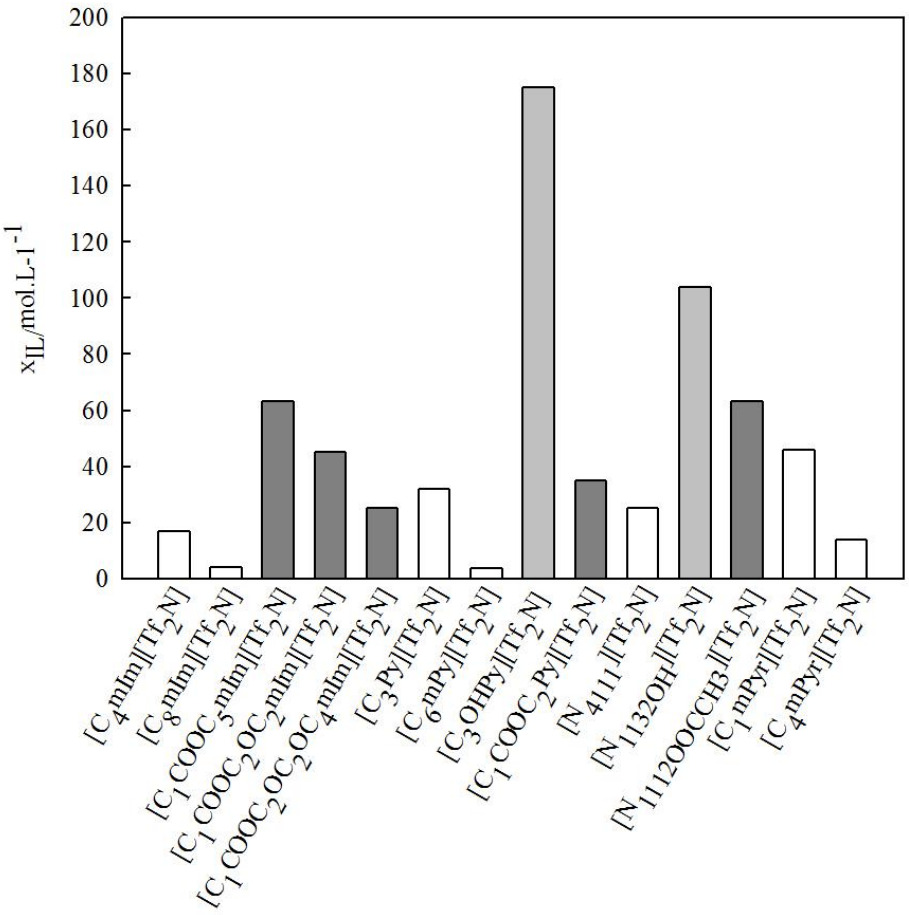

Figure 5.3 Aqueous solubility of ionic liquids at 22 ℃. The ionic liquids containing a ester group are in dark grey, the ones containing a hydroxyl group in light grey and the non-functionalised ones are in white.

5.3 Octanol-water partition coefficient

The K_{OW} values of all the tesed ionic liquids range from 0.01 to 3, which are similar to those of low-molecular weight polar solvents (except halogenated solvents), much lower than those of high molecular weight polar solvents and apolar solvents. They indicate that these ionic liquids have low possibility of accumulation in environment and are in favour of a minor environmental impact.

The ionic liquids containing longer apolar side chian on cation exibit a higher K_{OW} value. The presence of both ester and ether groups on the imidazolium and pyridinium cation decreases the value of their octanol-water partition coefficient, which means that the lipophilicity of this family of ionic liquids decreases when the alkyl side-chains contain these oxygen functionalities. The anions also affect K_{OW}.

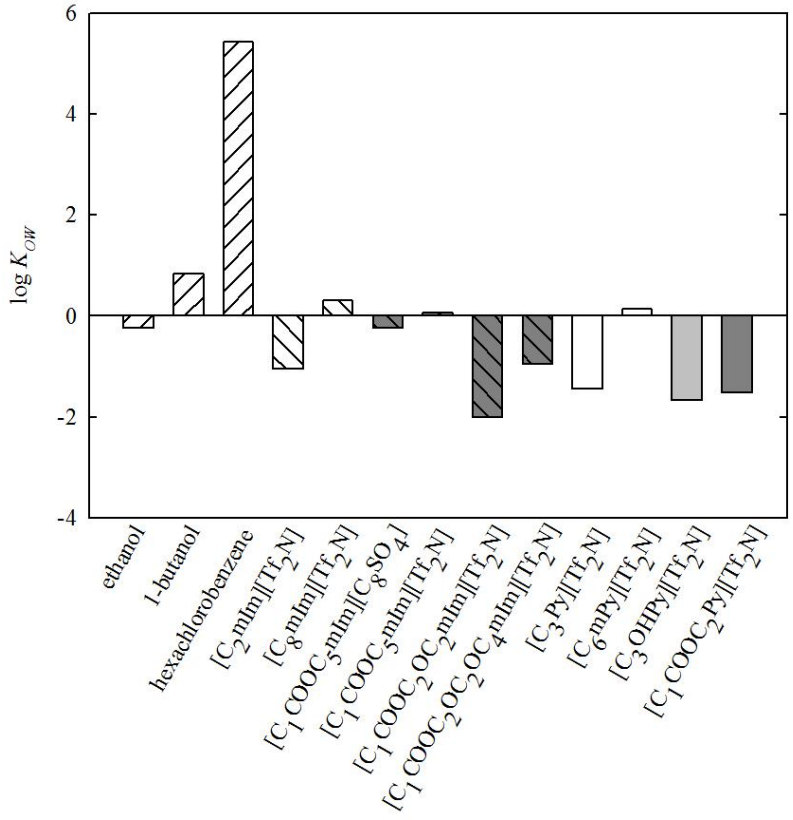

Figure 5.4 log K_{OW} of ionic liquids and some common solvents at 22-30 ℃. The chemicals that are not ionic liquids and ionic liquids not tested in this work are with hatching. The ionic liquids containing a ester group are in dark grey, the ones containing a hydroxyl group in light grey and the non-functionalised ones are in white.

5.4 Transport properties

A. Viscosity

The viscosities of all the studied ionic liquids decrease dramatically with temperature, as expected.

The ionic liquid is with the largest viscosity if the one containing the $[C_8SO_4]^-$ anion. The ester groups on the side chains of the cations – imidazolium, pyridinium or ammonium – greatly increased the viscosity whilst the hydroxyl group only slightly increased it.

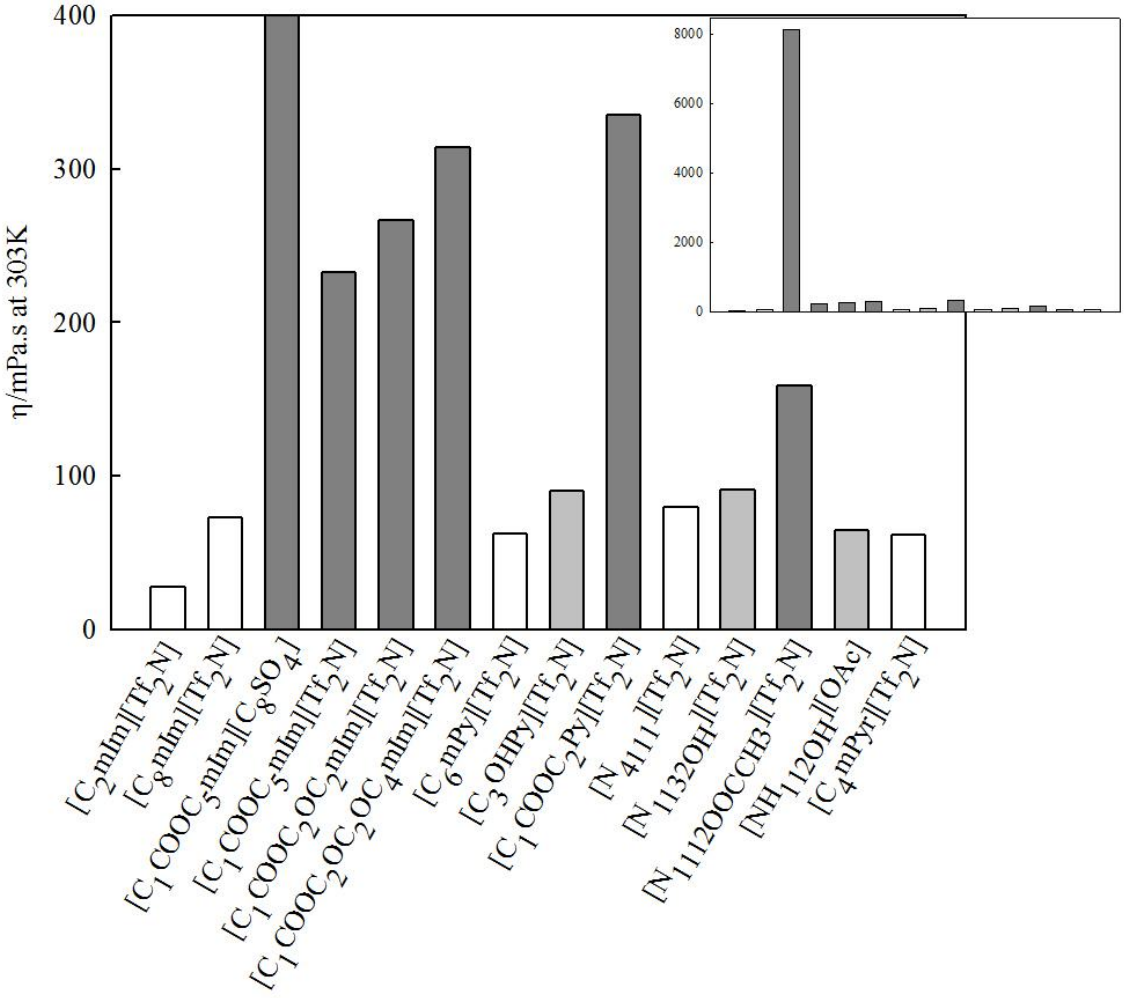

Figure 5.5 Viscosity of ionic liquids at 303K. The ionic liquids containing a ester group are in dark grey, the ones containing a hydroxyl group in light grey and the non-functionalised ones are in white.

B. Diffusion coefficient in water

The diffusion coefficient in water of all the ionic liquids do no differ obviously: the coefficient decreased with molecular weight. This correlation is almost linear for the ionic liquids with the same cation, the introduction of functional groups causing no influence. The coefficients of ionic liquids are higher than the polyethylene with a closed molecular weight (400) but lower than the classic organic chemicals such as acetronitrile.

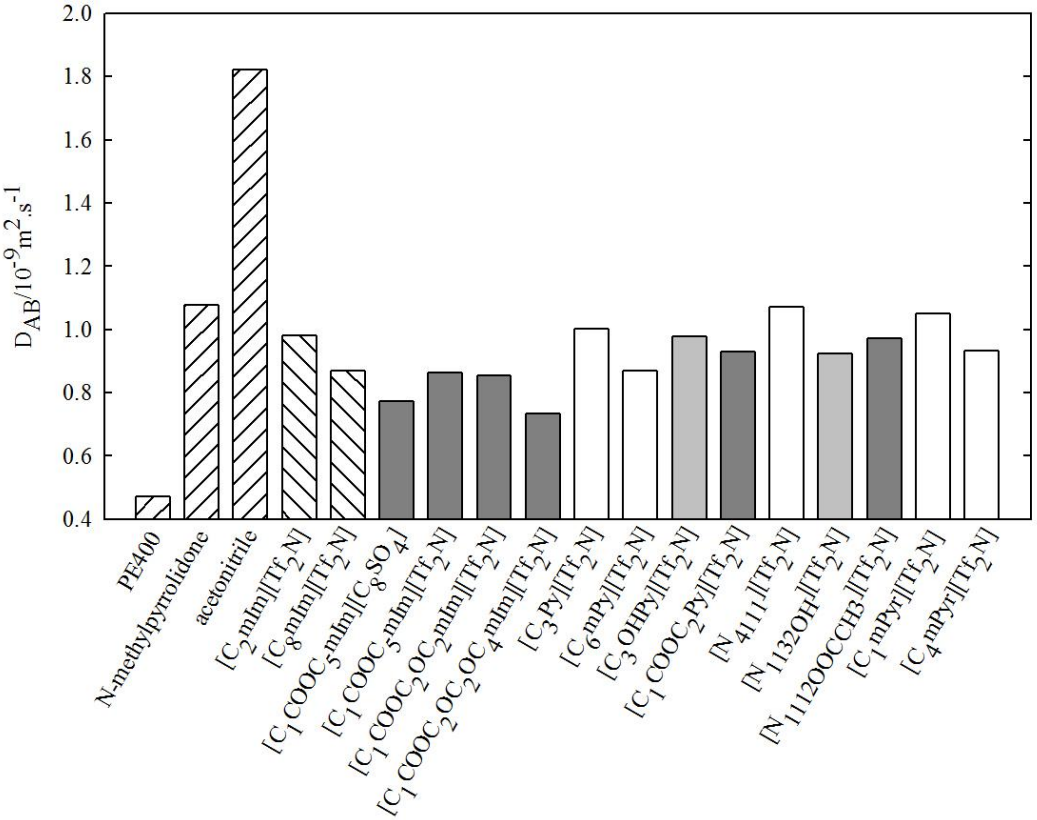

Figure 5.6 Infinite dilution diffusion coefficient of ionic liquids in water at 303K. The chemicals that are not ionic liquids and ionic liquids not tested in this work are with hatching. The ionic liquids containing a ester group are in dark grey, the ones containing a hydroxyl group in light grey and the non-functionalised ones are in white.

The models using molecular weight or Van der Walls Volumes as descriptor to predict the diffusion coefficient of $[Tf_2N]^-$ based ionic liquids with an estimated uncertainty lower than 5%. But the ionic liquids with $[OAc]^-$, $[C_8SO_4]^-$, and some other ions in the literatures are more disperse.

5.5 Toxicity

The minimum inhibitory concentrations (MIC) of ionic liquids are listed in Table 5.1. The test was towards four bacteria (*B.c. Bacillus cereus* ATCC 14579 and *R. r. Rhodococcus rhodochrous* ATCC 29672 as Gram positive bacteria, *P. a. Pseudomonas aeruginosa* ATCC 17504 and *E. c. Escherichia coli* ATCC 11303 as Gram negative bacteria) and one yeast (*C. a. Candida albicans* CIP 444).

Table 5.1 Minimum inhibitory concentrations (MIC) of ionic liquids (mmol.L^{-1}).

	B. c. (+)	*P.a*(-)	*E. c*(-)	*R. r.* (+)	*C. a.*
$[C_6mIm][Tf_2N]$	2	> 2	1	1	> 2
$[C_8mIm][Tf_2N]$	1.4	> 1.4	0.7	0.3	1.4
$[C_1COOC_5mIm][C_8SO_4]$	3.8	7.5	3.8	7.5	3.8
$[C_1COOC_5mIm][Tf_2N]$	> 5.5	> 5.5	5.5	5.5	> 5.5
$[C_1COOC_2OC_2mIm][Tf_2N]$	20.3	> 20.3	10.1	10.1	> 20.3
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	> 6.4	> 6.4	6.4	6.4	> 6.4
$[C_1COOHmIm][Cl]$	> 23	> 23	> 23	> 23	> 23
$[C_3Py][Tf_2N]$	15	>15	3.8	15	> 15
$[C_6mPy][Tf_2N]$	1.3	> 1.3	0.3	0.6	> 1.3
$[C_3OHPy][Tf_2N]$	> 15	> 15	15	> 15	> 15
$[C_1COOC_2Py][Tf_2N]$	15	> 15	15	15	> 15
$[N_{4111}][Tf_2N]$	> 12.5	> 12.5	6.3	> 12.5	> 12 5
$[N_{1132OH}][Tf_2N]$	45	> 45	22.5	45	> 45
$[N_{1112OOCCH3}][Tf_2N]$	> 30	> 30	15	> 30	> 30
$[NH_{112OH}][OAc]$	> 47.5	> 47.5	> 47.5	47.5	> 47.5
$[C_1mPyr][Tf_2N]$	> 12	> 12	12	> 12	> 12
$[C_4mPyr][Tf_2N]$	> 2	> 2	> 2	> 2	> 2

For tested ionic liquids in all the cation families, differences were observed between strains, but no relation with the cell wall types was observed: *E. coli* is the most sensitive and *P. aeruginosa* is the most resistant in most of the cases, whereas both are Gram-negative.

Overall, the studied ionic liquids are not very toxic for the strains tested, mostly with MIC superior to their aqueous solubilities. Myles *et al*. have measured the MIC of ionic

liquids $[C_1COOC_{4,\ 6\ or\ 8}\ mIm]^+$ with $[Br]^-$ and $[BF_4]^-$ anions, on bacteria and fungi.[128] The results are all superior to 2 mmol.L^{-1} and they claim those ionic liquids are non-toxic. In our study, the imidazolium-based ionic liquids containing ester and/or ether groups, as well as the ionic liquids with other cations show the similar low toxicity.

Among all the studied ionic liquids, the imidazolium-based ones present relatively higher toxicity than the ionic liquids containing other types of cations. The imidazolium and pyridinium based ionic liquids with long alkyl chains, $[C_8mIm]^+$ and $[C_6mPy]^+$ show highest toxicity towards the microorganisms. The ammonium based ones showed the lowest toxicity (Figure 5.7).

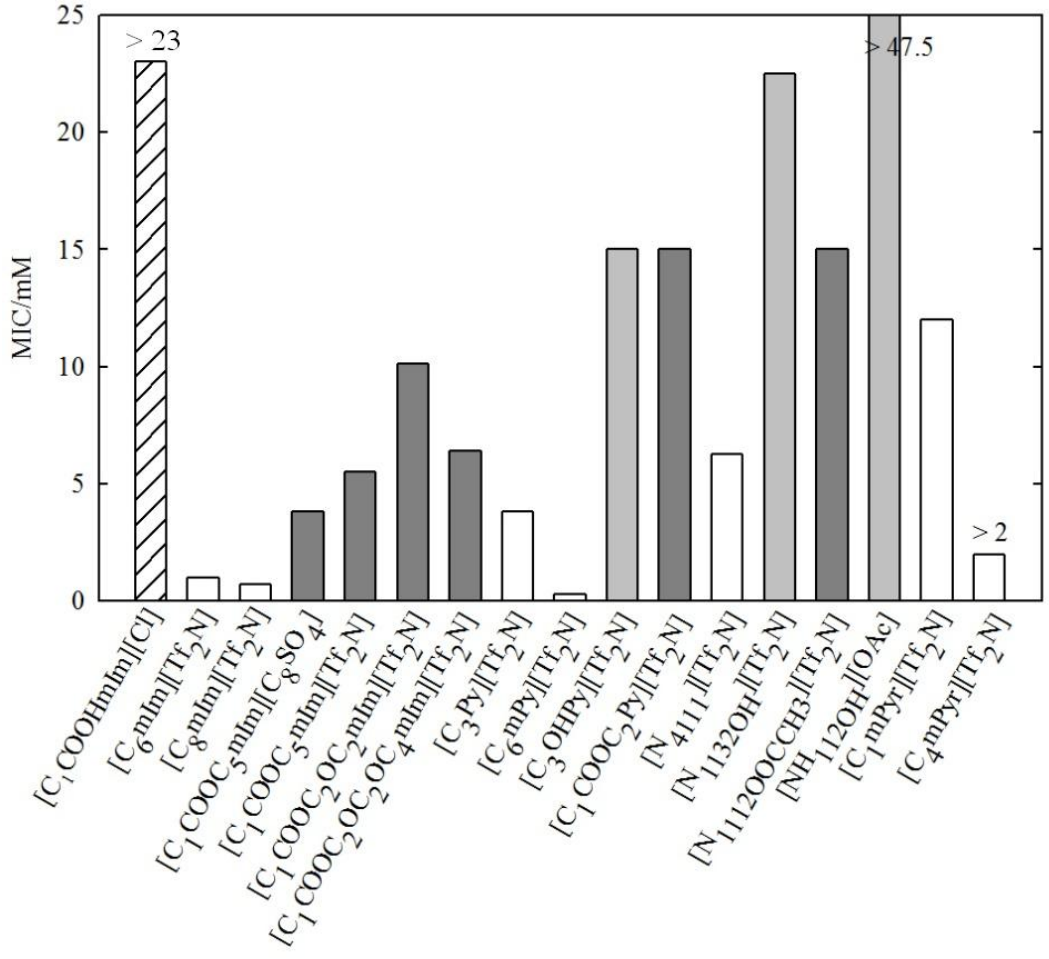

Figure 5.7 MIC of ionic liquids towards *E. coli* ATCC 11303. The chemical that are not ionic liquids is with hatching. The ionic liquids containing a ester group are in dark grey, the ones containing a hydroxyl group in light grey and the non-functionalised ones are in white.

The MIC of $[C_8mIm][Br]$ on *C. albicans* KCTC1940 and *E. coli* KCTC1924 were 1 $mmol.L^{-1}$ and 0.3 $mmol.L^{-1}$ respectively in the study of Demberelnyamba *et al.*[130] These values are a little lower than our results concerning the same cation, but in the same magnitude.

Within the same cation family, the dependence between toxicity and the length of apolar alkyl chain on cations is very obvious. The presence of oxygenated groups in the alkyl chains reduced the toxicity, which is also observed by other authors.[122]

In the case of $[C_1COOC_5mIm]^+$ the anion $[C_8SO_4]^-$ is slightly more toxic than $[Tf_2N]^-$, This result differs from the study of Morrissey *et al.*[19] $[OAc]^-$ anion rends the ionic liquid less toxic.[230]

5.6 Biodegradability

The long alkyl chain ionic liquids, $[C_6mIm]^+$, $[C_8mIm]^+$ and $[C_6mPy]^+$, were not degraded either under abiotic or biotic conditions (Table 5.2).

Table 5.2 Degradation studies of the ionic liquids under abiotic and biotic (*Rhodococcus rhodochrous* ATCC 29672) conditions.

	Abiotic conditions		Biotic conditions	
	Time	% of degradation	Time	% of biodegradation
$[C_6mIm][Tf_2N]$	49 days	0%	49 days	0%
$[C_8mIm][Tf_2N]$	49 days	0%	49 days	0%
$[C_1COOC_5mIm][Tf_2N]$	70 days	51%	4.5 hours	100%
$[C_1COOC_5mIm][C_8SO_4]$	70 days	53%	12.5 hours	100%
$[C_1COOC_2OC_2OC_4mIm][Tf_2N]$	70 days	89%	23 hours	100%
$[C_1COOC_2OC_2mIm][Tf_2N]$	70 days	81%	23 hours	100%
$[C_6mPy][Tf_2N]$	49 days	0%	49 days	0%
$[C_3Py][Tf_2N]$	49 days	0%	14 days	76%
$[C_3OHPy][Tf_2N]$	49 days	0%	28 days	85%
$[C_1COOC_2Py][Tf_2N]$	49 days	76%	28 hours	100%
$[N_{1132OH}][Tf_2N]$	49 days	0%	49 days	79%
$[N_{4111}][Tf_2N]$	49 days	0%	28 days	88%
$[N_{1112OOCCH3}][Tf_2N]$	49 days	24%	7 days	100%
$[NH_{112OH}][OAc]$	28 days	100%	1 day	100%
$[C_1mPyr][Tf_2N]$	49 days	0%	28 days	80%
$[C_4mPyr][Tf_2N]$	49 days	0%	28 days	85%

Their concentration increased with time in the presence of the microorganism, reaching the expected initial concentration only after several weeks. Adsorption or strong interactions with the cells can be suggested to explain this result. Docherty *et al*. have used activated sludge as inoculum and $[C_6mPy][Br]$ can be fully biodegraded.[13,143] This difference is due to the different microbial community existing in the inocula. The other non-functionalised ionic liquids with a shorter lateral chain were not degraded under abiotic conditions but all of them were biodegradated in the presence of *R. rhodochrous*. The percentage of biodegradatin did not vary much, and around 80-85% of the starting

material disappeared after 28 days of incubation ($[C_3Py]^+$ < $[C_1mPyr]^+$ < $[C_4mPyr]^+$ < $[N_{4111}]^+$). Ammonium and pyrrolidinium-based ionic liquids exhibited higher biodegradabilities than imidazolium and pyridinium ones and were completely degraded.

Some oxygenated functionalized ionic liquids were tested. The ionic liquids containing an ester group whatever their families were degraded under abiotic and biotic conditions leading to the formation of the hydrolysis products. The hydrolysis is much more rapid in the presence of microorganisms. This observation has been already reported by several studies.[2] The ammonium-based ionic liquids are degraded much slower than imidazolium and pyridinium-based ones. Nevertheless the presence of an ester group just made the chains on cation more susceptible to hydrolysis, but the imidazolium and pyridinium rings were always resistant to biodegradation under our conditions. The introduction of an ethereal moiety has also a negative effect on the global biodegradation rate (comparison of $[C_1COOC_5mIm][Tf_2N]$ and $[C_1COOC_2OC_2mIm][Tf_2N]$) but favours its hydrolysis under abiotic conditions.

Ionic liquids containing terminal hydroxyl groups were not degraded under abiotic conditions. $[N_{1132OH}]^+$ and $[C_3OHPy]^+$ showed similar rates of biodegradation with R. rhodochrous. From our study, the introduction of terminal hydroxyl group seems to favour the biodegradability. The initial step of terminal hydroxylation of the alkyl chain can occur due to the presence of molecular oxygen and monooxygenase, leading to the corresponding carboxylic acid analogue via ω-oxidation mechanism. This degradation product is then submitted to β-oxidation.

The global biodegradation of $[C_1COOC_5mIm]^+$ was influenced by the nature of the anion moiety ($[C_8SO_4]^-$ *vs* $[Tf_2N]^-$), the degradation was decreased by a factor 4 in presence of the $[C_8SO_4]^-$.

In conclusion, the non-aromatic ionic liquids are more easily biodegradable than the aromatic ones. The introduction of an ester groups in the alkyl chain of all types of cations improves the biodegradability of the alkyl side chains, but the imidazolium and pyridinium rings are not mineralised. The presence of hydroxyl group generally improves the biodegradability of ionic liquids but sometimes only slightly (ammonium-based ionic liquid).

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